MANUSCRIPT 2

SALT CREEK *IN SITU* TOXICITY TESTING PROGRAM

CITY OF LINCOLN, NEBRASKA
SALT CREEK WATER QUALITY STUDIES

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SALT CREEK *IN SITU* TOXICITY TESTING PROGRAM

Key Findings

The following are Key Findings from Salt Creek *in situ* Toxicity Testing Program which highlight the main points and conclusions. Extensive detail of the *in situ* program, final results and final conclusions are also presented within this Manuscript.

- Test species selection was based upon (1) the availability of an appropriate test methodology for application to Salt Creek, (2) acute and chronic sensitivity to ammonia (U.S. EPA 1998), and (3) species which could be cultured and available in suitable numbers and lifestages.

- An *in-situ* toxicity test was performed in which groups of caged fish (fathead minnows and channel catfish) were continuously exposed to ammonia for 30 days at nine locations in Salt Creek: Three “reference” sites upstream of both wastewater treatment plants, one site immediately upstream of the Northeast Wastewater Treatment Plant (WWTP), and five sites downstream of the Northeast WWTP.

- The fathead minnows were unaffected, with the 30-day IC20 value above the highest in-stream total ammonia exposure concentration (> 9.98 mg-N/L).

- The 30-day IC20 total ammonia value for the channel catfish was 3.9 mg-N/L, which is the concentration of total ammonia that had a 20 percent effect on biomass (the combination of growth and survival).

- The *in-situ* results are “key” to determining a concentration of total ammonia, which will be protective of Salt Creek biological communities under chronic conditions.

1.0 INTRODUCTION

To more accurately characterize the complex interactions of ammonia in Salt Creek waters, an *in situ* toxicity test was performed in which caged fish were continuously exposed for 30-days at nine in-stream locations. The Work Plan for the *in situ* program was originally presented in the City's June 4, 1998 Work Plan, and then revised in the City of Lincoln (City) Technical Addendum #1 (dated 21 June 99). Addendum #1 was intended to be a stand-alone summary of the technical aspects of the *in situ* program, and to present an *á priori* approach to data integration. The *in situ* program was developed by the City and their consulting team (Brown & Caldwell; EA Engineering, Science & Technology, Inc.; and CH2MHill), with technical input from the Water Environment Research Foundation's (WERF) Peer Review Panel. Addendum #1 served as the study design for the *in situ* program and was submitted to, and reviewed by, the WERF Peer Review Panel and Nebraska Department of Environmental Quality (NDEQ) staff.

Understand that the *in situ* studies described are just one of several components of the Salt Creek Water Quality Studies (SCWQS) program that the team has been conducting since 1989. Other components are described in other manuscripts of this report (e.g., bio-assessment studies, chemistry and toxicology studies, and water quality modeling).

Although the final *in situ* program was carried out in the fall of 1999, extensive preliminary research preceded this effort. Specifically, an experimental test protocol was developed for *in situ* testing with channel catfish during 1997. The test protocol was then refined in a three-phase study conducted from July through October 1997. Documentation of this study was presented in Attachment C of the City's June 4, 1998 Work Plan.

- Phase 1, conducted in the laboratory, involved the design and development of the protocol, including selecting a test species, designing test chambers, and developing test procedures.

- Phase 2 was a "pilot" *in situ* study to implement and refine the methods developed in the laboratory, and to determine if the test fish would show significant growth during a two-week exposure period. From this study, modifications were determined and the *in situ* test procedure was adjusted accordingly.

- Phase 3 involved laboratory and field studies to determine the suitability of fathead minnows for the proposed *in situ* toxicity-testing program.

1.1 Overview of the In situ Program

The following *briefly* discusses the *in situ* testing procedures which were based on: (1) the methods development work completed in 1997; (2) discussions among Lincoln's technical consulting team; and (3) interaction with the WERF Peer Review Panel for the Lincoln project. Details are presented in the City's Technical Addendum #1.

1.1.1 General Location for the *in situ* Experiments

The area below the Northeast WWTP was identified for the *in situ* testing for three reasons: (1) in-stream ammonia concentrations are generally higher as required to meet the necessary chronic toxicity exposure range; (2) Salt Creek is deeper below the Northeast WWTP, which is necessary to properly locate the *in situ* testing chambers; and (3) the chemistry and WET test data for the Northeast WWTP shows less variability, which enhances the quality of the research. This information was presented to the WERF Peer Review Panel who agreed with the site location recommendation.

1.1.2 Test Species: Fathead Minnow and Channel Catfish

Species selection was based upon (1) the availability of an appropriate test methodology for application to Salt Creek, (2) acute and chronic sensitivity to ammonia (U.S. EPA 1998), and (3) species which could be cultured and available in suitable numbers and lifestages. Species recommendations were presented to and discussed with NDEQ staff, as well as the WERF Peer Review Panel before the *in-situ* program was initiated. *Ceriodaphnia* and *Daphnia* were eliminated from consideration because research showed they would not survive normally encountered Salt Creek salinity, and there was no workable chronic testing exposure apparatus.

To assess the effects of ammonia, two fish species were selected: fathead minnows (*Pimephales promelas*) and channel catfish (*Ictalurus punctatus*).

- According to the U.S. EPA's 1998 update of ambient water quality criteria for ammonia, the fathead minnow is one of the most sensitive species to ammonia with a Species Mean Chronic Value (SMCV) of 3.09 mg N/L total ammonia (U.S.EPA 1998). The summer site-specific criterion in Segment LP2-20000 is in the same range at normal pH values, and the species is the most likely resident Salt Creek species to be impaired by exceedences of the criteria.

- The channel catfish is defined as a "key species" in Salt Creek (Title 117; 4:003.01G1). With a SMCV of 8.84 mg N/L total ammonia, it is not among the more sensitive species in U.S. EPA's (1998, 1999) chronic toxicity database, but is a resident species and is salinity tolerant.

An advantage of using fathead minnows as a primary test species is that organisms can be obtained from a disease-free laboratory-reared population, and a large number of same age (within a narrow age window) cohorts can be produced which minimizes age and size differences at test initiation.

1.1.3 Ammonia Effect Levels and Predicted Salt Creek Concentrations

The location of *in situ* testing fish cages was based on *target* total ammonia concentrations (and salinity) in Salt Creek. The target ammonia concentrations needed to include a chronic effect level for a 30-day exposure period to fathead minnows, so that the test results could be used to develop a 30-day EC20 value[1]. Based on the 1998 Addendum to the U.S.EPA (1984) Ammonia Criteria Document, the chronic effect level is approximately 3.09 mg N/L total ammonia for the fathead minnow, and 8.84 mg N/L for the channel catfish. To develop an EC20 value, or an alternative endpoint that might better predict chronic threshold effect concentrations to resident communities, *in situ* test cages were placed in Salt Creek at locations covering a range of in-stream total ammonia concentrations. The locations of the test cages were chosen to bracket the range of EC20 concentrations that have been reported for total ammonia ranging from < 3 mg N/L to ~10 mg N/L total ammonia. Note that to obtain sufficiently high concentrations to have a chronic effect, the City's Northeast WWTP was operated in such a way that end-of-pipe ammonia concentrations were substantially higher than normal.

1.1.4 Location of *In Situ* Testing Chambers

The selection of *in situ* fish testing cage locations below the Northeast WWTP were based on two factors:

- total ammonia concentrations, to address the chronic toxicity of ammonia; and
- salinity, to account for potential mitigative effects.

[1] An EC20 value is the concentration of a toxicant (e.g., ammonia) which causes a 20 percent effect in a toxicological endpoint (e.g., mortality, biomass) relative to an appropriate experimental control. The EC20 value was the measure used in U.S. EPA's (1998, 1999) revised ammonia criteria documents.

For this *in situ* ammonia study, five locations downstream of the Northeast WWTP were used (Stations E, F, G, H and I), in addition to one ammonia "control" station immediately upstream from the Northeast WWTP (Station D). To evaluate the effect of salinity, three additional upstream salinity "controls" were located above the Theresa Street WWTP (Stations A, B, and C) to reflect the potential range of salinities at the downstream stations. This experimental design resulted in a total of nine stations (Figure 2-1).

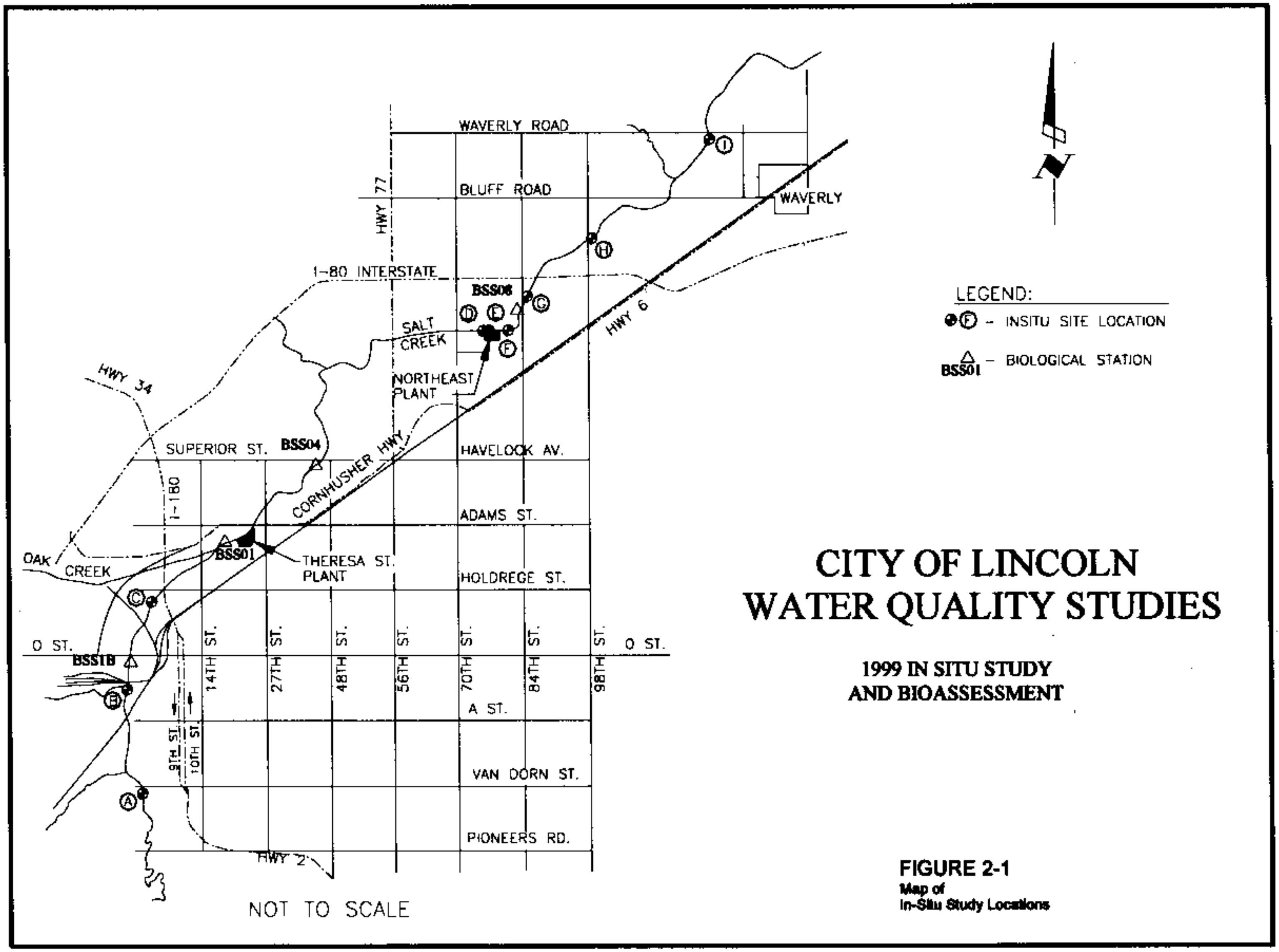
1.1.5 *in situ* Testing Sample Size Requirements

Sample size requirements for *in situ* testing were defined to detect differences between two populations with equal variances and account for the site-specific application to Salt Creek. A detailed analysis of the required sample size, discussing the theoretical aspects of sample size criteria, and the application of site-specific information from Salt Creek to determine an appropriate sample size, was provided in the City's June 4, 1998 Work Plan. In general, the sample size required at each *in situ* site was based on data collected from the catfish growth study from the Salt Creek *in situ* pilot testing. Note that the final program was *increased* from 14 days to a 30-day exposure period, which should theoretically increase the precision of the EC20 endpoint.

The replicate sample size was 8 test chambers per site (per species) with 15 fish (fathead minnows or channel catfish) in each chamber. This design was expected to allow the detection of a 20 percent difference in growth among sites (during a 30-day test) with a 90 percent confidence.

Note that because of the possibility of severe weather and high flows that could threaten the entire field program, a decision was made by the project team to deploy a total of 32 chambers *per site* (16 chambers for fathead minnows and 16 for channel catfish). This approach allowed for half of the chambers (8) for each species to be "harvested" and yield a valid test anytime after 15 days, leaving the remaining 16 chambers in stream for the full 30 days.

Figure 2-1 Location of *in situ* Testing Chambers



1.1.6 Chemical Monitoring

Extensive chemical monitoring was conducted both in Salt Creek and within the Northeast and Theresa Street WWTP's final effluent prior to and during *in situ* testing in Salt Creek. Water quality parameters monitored included: total ammonia, chloride, pH, conductivity, temperature, creek flow and velocity, air temperature and precipitation. Details on the locations and frequency of these water quality measurements are presented in this document.

In addition, during *in situ* testing, limited pesticide and herbicide sampling was conducted to evaluate other significant factors that could affect the *in situ* test results. Pesticide and herbicide samples were collected at three times during the 30-day test at three locations: (1) upstream of the Theresa Street WWTP at *in situ* location C; (2) just above the Northeast WWTP at *in situ* location D; and (3) below the Northeast WWTP at *in situ* location I.

1.1.7 Additional QA/QC Testing for *In Situ* Program

Extensive efforts were conducted to ensure that data quality would be acceptable in a research/regulatory program of this type. These efforts included:

- salinity tolerance testing of 30- to 45-day old fathead minnows and channel catfish;
- salinity intolerance testing of < 24-hour old fathead minnow larvae;
- test organism age study to quantify the sensitivity of < 24-hour old fathead minnows versus 30- to 45-day old fathead minnows to ammonia;
- examination of the quality of test organisms using reference toxicant testing;
- parallel chronic WET testing (during *in situ* program);
- an *in situ* salinity tolerance study using a series of control stations in several salinity zones (e.g., 1, 2, and 4 ppt, located upstream of the Theresa Street WWTP);
- dye testing of exposure chambers to quantify the turnover volumes to ensure there was sufficient flow through the chambers; and
- numerous field QA/QC issues including randomizing cage locations, cage maintenance staff and staff responsibilities within each team.

Details on each of the above are presented in the City's Technical Addendum #1.

Comprehensive sampling and testing for total ammonia, chlorides, conductivity, water temperature, pH, and dissolved oxygen was accomplished by the City at each *in situ* site during the 30-day testing period. For the total ammonia testing, there were 1,939 tests completed, of which 11.9% were sample duplicates, and 1.4% were field blanks, for a total QA/QC effort of 13.3% of all ammonia tests. Similarly, there were 1,793 chloride tests run with 7.5% QA/QC sample duplication. In addition, continuous data logging with HydroLab® units was supplemented by portable meter independent spot checks.

1.2 Station Locations

The locations of the *in situ* study sites are described in Table 2-1A. Downstream stations were selected to allow for the range of ammonia exposure concentrations necessary to calculate chronic EC20 values. Upstream stations were selected for two important goals: (1) to reflect a range of chloride concentrations to examine the effect of "salinity" on fish growth and survival; and (2) yield an appropriate experimental "control" for the determination of EC20 values. Stations A, B and C are upstream of the City's wastewater treatment plants, and contain only background concentrations of ammonia. Station D is downstream from the Theresa Street WWTP and immediately upstream of the Northeast WWTP outfall. Stations E, F, G, H and I extend downstream from the Northeast WWTP outfall, and are the focus of the *in situ* experiment. Table 2-1B shows the water quality parameters that were measured at each of these nine stations, as well as at the two WWTP outfalls.

Table 2–1A. Description of Sites used in the October, 1999 Salt Creek *In Situ* Study

Site Name	Description
A	Reference site upstream of Haines Branch confluence. Conductivity <1000 µS/cm and ammonia concentrations at background levels.
B	Reference site, conductivity same range as downstream sites (4,000 – 5,000 µS/cm). Ammonia concentrations at background levels.
C	Reference site, conductivity 6,000 – 7,000 µS/cm, ammonia concentrations at background levels. Upstream of the Theresa Street WWTP.
D	Immediately upstream of the Northeast WWTP outfall, control site for effects from Theresa St. outfall, Goodyear ditch, etc. Had elevated ammonia concentrations during study (5–7 mg N/L)
E	The site with highest ammonia concentrations. Approximately 70 meters downstream of the Northeast WWTP outfall.
F	Approximately 600 m downstream of the Northeast WWTP outfall, upstream confluence with Stevens Creek
G	84th Street Bridge approximately 1,500 meters downstream of the Northeast outfall
H	98th Street Bridge approximately 3,645 m downstream of the Northeast WWTP outfall. No fathead minnows at this site.
I	Approximately 8,000 meters downstream from the Northeast WWTP outfall located at Waverly Road.
Theresa Street WWTP outfall	WWTP between Stations C and D
Northeast WWTP Outfall	WWTP just downstream of Station D
USGS 27th St. (Site 3)	USGS gauging station located near 27th Street Bridge. Velocity/flow transects and chemical sampling were also conducted at this site for studying relationships with the Theresa St. Treatment Plant effluent.
USGS 70th St.	USGS gauging station located at 70th Street Bridge

Table 2–1B. Summary of the Water Quality Data Collected during the October, 1999 Creek *In Situ* Study

Water Quality Parameters	Location	Collection Frequency
flow, velocity	*in situ* Stations, WWTP outfalls, Site 3, and USGS gauging stations	Continuous metering at WWTP outfalls and USGS gauging stations; daily transects for *in situ* stations D, F, G, and I and Site 3.
air temperature, precipitation	*in situ* Stations, WWTP outfalls	Continuous metering at University of Nebraska High Plains Climate Center weather station sites.
pH, conductivity, temperature, dissolved oxygen	*in situ* Stations and Site 3	Continuous metering (except for dissolved oxygen) with HydroLab® units at all *in situ* stations; portable meter spot checks twice per day at *in situ* stations A, B, and C and Site 3 and once per day at *in situ* stations D–I.
Total Ammonia and chlorides	*in situ* Stations, WWTP outfalls	Every 4 hours for *in situ* stations D–I and twice daily at *in situ* stations A, B, and C.

2.0 FISH MORTALITY EVALUATION

Each exposure chamber at each site was evaluated twice per day during feeding and maintenance activities throughout the 30-day exposure period, and mortalities in each chamber were recorded in bound logbooks for each station. In addition, to help ensure that organism exposure was not influenced by chamber location relative to the other *in situ* exposure chambers, the position of the chambers within the array at each site was changed daily. Chamber positions within each array were numbered (1 through 32) for each of the *in situ* study sites. Chambers located in an odd position were rotated daily downstream to the next odd position and likewise with chambers occupying even numbered positions. Thus, a chamber at position 1 at the initiation of the study would have occupied every odd position in the array at the end of the fifteen-day test (see Section 2.2).

The fathead minnow and channel catfish mortality data for the first 15 days are presented in Figure 2-2, and the data for those organisms exposed for the full 30 days are presented in Figure 2-3. The numeric values plotted in these figures are in Tables 2-2A and 2-2B.

2.1 15-Day Exposure Results

For the first 15 days (Figure 2-2), mortality upstream and downstream of the Northeast WWTP were low. The mean percent mortality for fathead minnows was 3.2 percent (Table 2-2A; n = 8), and the mean value for channel catfish was 0.5 percent (n = 9). Further, the limited mortality that did occur during this period appears unrelated to ambient ammonia concentrations (e.g., upstream Stations A and B have the highest mortality for fathead minnows).

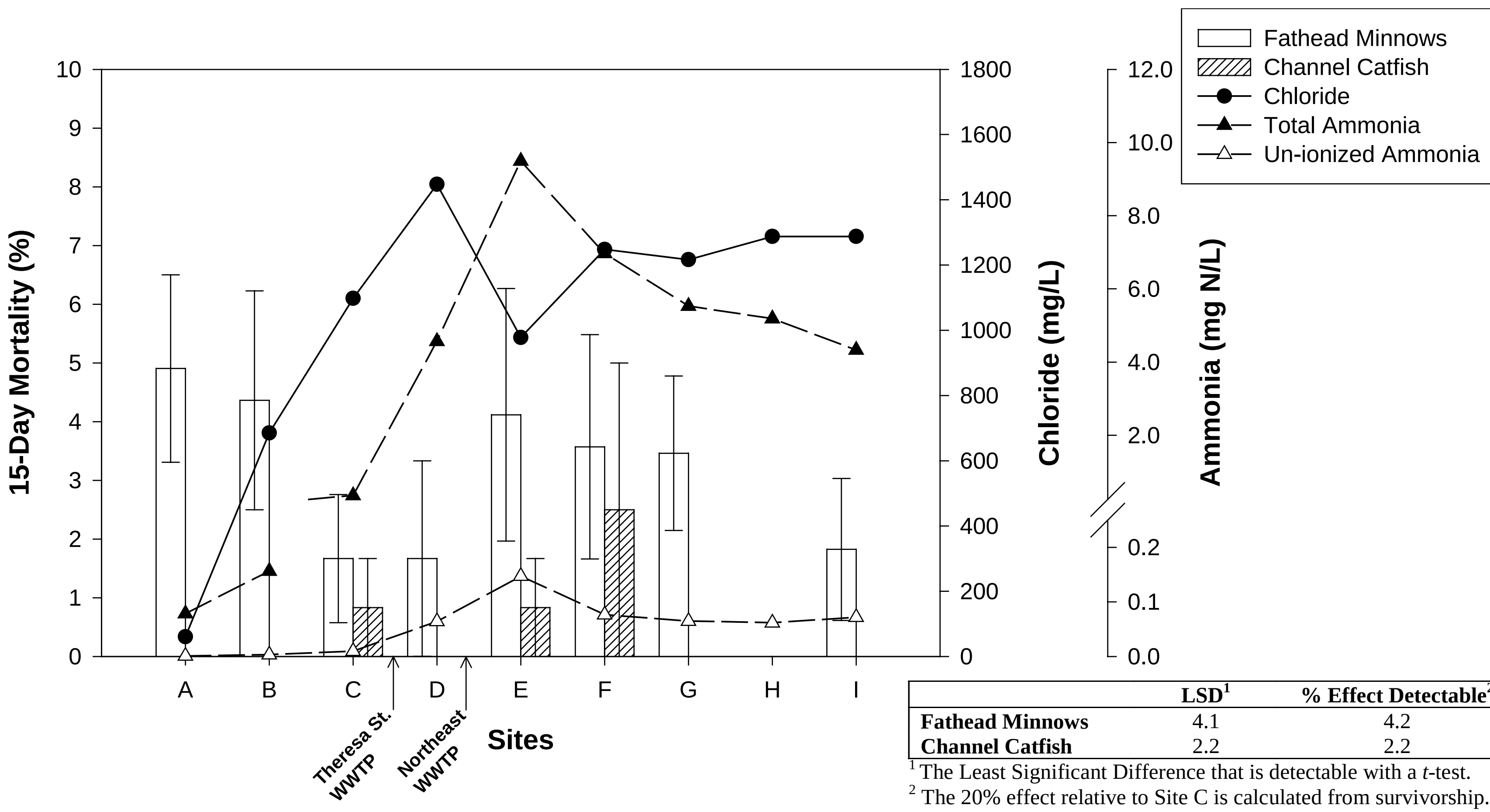
2.2 30-Day Exposure Results

The 30-day dataset is shown in Figure 2-3 and Table 2-2B. Mean mortality for the fathead minnows averaged 6.8 percent for the eight stations, with the highest mortalities at upstream stations A and B, and downstream stations E and F (Table 2-2B). The channel catfish data are more difficult to explain. There was almost no mortality at any of the stations except at Stations E and F, which are immediately downstream of the Northeast WWTP outfall. At those two stations total mortality was 42.0 and 44.8 percent respectively, and mortalities occurred on several different dates[2]. Note, however, that the majority of the mortality at Stations E and F occurred at only a few chamber positions.

[2] At Station E, catfish mortalities occurred on 9-26 (7 fish), 9-27 (10), 10-8 (15) and 10-10 (14 fish). At Station F, catfish mortalities occurred on 10-7 (6 fish), 10-8 (15), 10-10 (13), and 10-12 (12). Expanded water quality studies at Stations E and F were conducted on 10 October 99.

Figure 2-2   Summary of the 15-Day Fish Mortality

The 15-Day fish mortality (±1 SE, n = 8). Mortality rates for the stations downstream of the WWTPs were not significantly different from the upstream control site C, as determined by pairwise one-tailed *t*-tests (H_0: $\bar{X}_C \geq \bar{X}_i$; $\alpha = 0.05$). Average chloride and both total and un-ionized ammonia concentrations are superimposed. See Appendix 2 for additional descriptions of water chemistry.



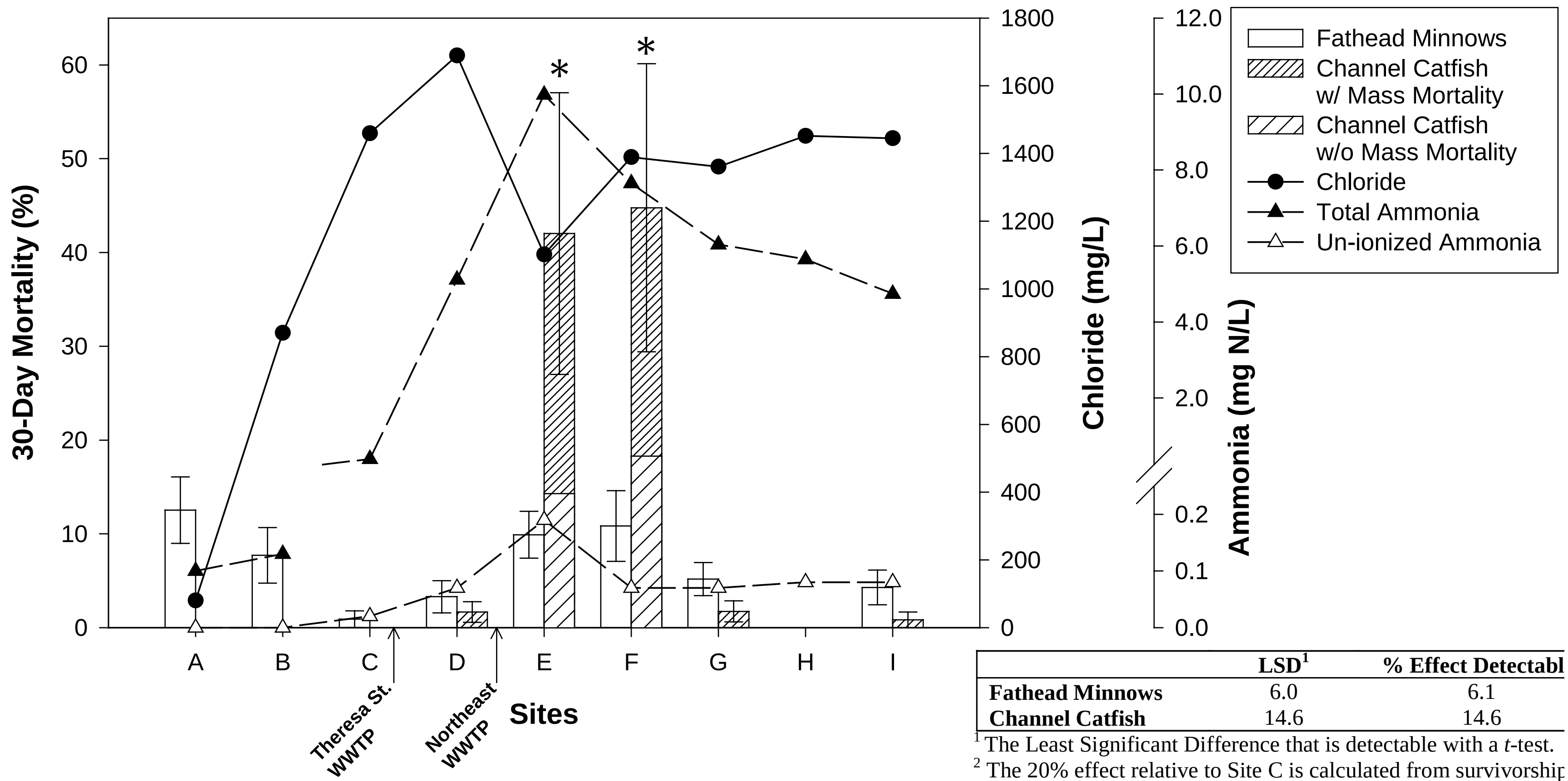
	LSD[1]	% Effect Detectable[2]
Fathead Minnows	4.1	4.2
Channel Catfish	2.2	2.2

[1] The Least Significant Difference that is detectable with a *t*-test.

[2] The 20% effect relative to Site C is calculated from survivorship.

Figure 2-3 Summary of the 30-Day Fish Mortality

The 30-Day fish mortality (±1 SE, n = 8). Asterisks (∗) indicate the stations downstream of the WWTPs that show significant impairment relative to the control Site C, as determined by pairwise one-tailed *t*-tests (H_0: $\overline{X}_C \geq \overline{X}_i$; $\alpha = 0.05$). Stacked bars in Channel Catfish sites E and F show the relative contribution of unexplained mass mortality (>50% mortality in 24-hours). Average chloride and both total and un-ionized ammonia concentrations are superimposed. See Appendix 2 for additional descriptions of water chemistry.



	LSD[1]	% Effect Detectabl
Fathead Minnows	6.0	6.1
Channel Catfish	14.6	14.6

[1] The Least Significant Difference that is detectable with a *t*-test.
[2] The 20% effect relative to Site C is calculated from survivorshi

Table 2–2A. The 15-Day Fish Mortality

Site	Fathead Minnow			Channel Catfish		
	% Mortality	(SE)	n	% Mortality	(SE)	n
A	4.9	(1.6)	8	0.0	(0.0)	8
B	4.4	(1.9)	8	0.0	(0.0)	8
C	1.7	(1.1)	8	0.8	(0.8)	8
D	1.7	(1.7)	8	0.0	(0.0)	8
E	4.1	(2.2)	8	0.8	(0.8)	8
F	3.6	(1.9)	8	2.5	(2.5)	8
G	3.5	(1.3)	8	0.0	(0.0)	8
H	-	-	-	0.0	(0.0)	8
I	1.8	(1.2)	8	0.0	(0.0)	8

Table 2–2B. The 30-Day Fish Mortality

Site	Fathead Minnow			Channel Catfish		
	% Mortality	(SE)	n	% Mortality	(SE)	n
A	12.5	(3.6)	8	0.0	(0.0)	8
B	7.7	(3.0)	8	0.0	(0.0)	8
C	0.9	(0.9)	8	0.0	(0.0)	8
D	3.3	(1.7)	8	1.7	(1.1)	8
E*	9.9	(2.5)	8	42.0	(15.0)	8
F*	10.8	(3.8)	8	44.8	(15.4)	8
G	5.2	(1.8)	8	1.7	(1.1)	8
H	-	-	-	0.0	(0.0)	8
I	4.3	(1.8)	8	0.8	(0.8)	8
Neglecting unexplained mass mortality:						
E	NA			14.3	(5.0)	8
F	NA			18.3	(5.0)	8

As shown in Figure 2-4, the channel catfish mortalities at Station E occurred almost exclusively when the exposure chambers were in positions 18, 19 and 23 (and *not* after they were rotated out of this position). Chambers immediately in front of and behind these positions exhibited almost no mortality, and fathead minnows located in these *same* three positions (18, 19 and 23) showed no mortality. If the mortality from the specific Station E chambers that experienced the mass mortality are removed from the dataset, the mortalities at Station E are reduced from 42 percent down to 14.3 percent (Table 2-2B).

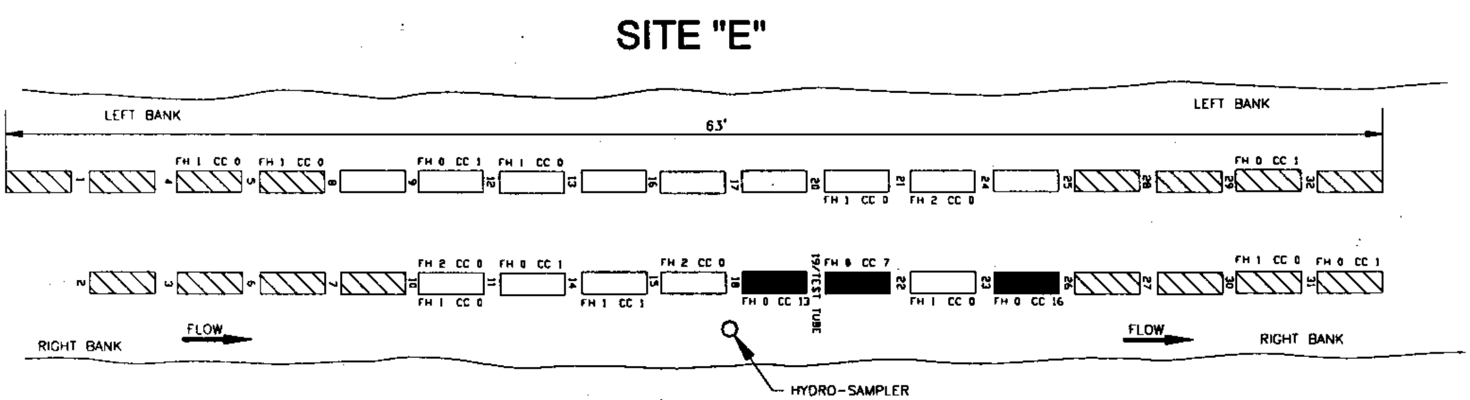
The same phenomenon was seen for channel catfish at Site F (Figure 2-5 and Table 2-2B). As shown in Figure 2-5, the channel catfish mortalities at Station F occurred almost exclusively when the exposure chambers were in two positions (7 and 9) and *not* after they were rotated to other Site F positions. Chambers immediately in front of and behind positions 7 and 9 exhibited almost no mortality, and fathead minnows located in these same two positions (7 and 9) showed almost no mortality. If the mortality from those two Site F chamber positions are removed from the dataset, the mortalities at Site F are reduced from 44.8 percent down to 18.3 percent (Table 2-2B).

On 8 October 1999 mass mortalities occurred at *in situ* Station E (position 23) and at *in situ* Station F (position 7). These channel catfish (15 per location) were stored on ice and delivered to the University of Nebraska Lincoln, Department of Veterinary & Biomedical Sciences, Veterinary Diagnostic Center for histopathological examination. Microscopic examination of tissue revealed no lesions or physical abnormalities, which would be indicative of viral or bacterial infections. Gross examination only revealed a tenacious mucous adhered to the gills.

Additionally, four channel catfish that had survived the 30 day *in situ* study—two survivors from the mass mortality chambers, one from a non-mortality chamber (location E), and one from upstream Station B—were sent to the University of Montana for histopathological analysis by Mr. Charlie Smith. Per the 4 February 00 memo from D. Rosenthal (Nebraska Game and Parks Commission) to L. Christensen (City of Lincoln), Mr. Smith reported no appreciable difference in the samples except for sample number E-CC-4 where he saw several parasitic worms in the intestine. He also reported seeing some *Henneguya* cysts on the gills and integument of some fish, but no great differences (between the specimens).

To supplement these pathological examinations, more detailed studies were conducted on the morning of 8 October in the vicinity of these chamber positions to help determine if ammonia or other water quality parameters might explain these mortalities. As shown in the imbedded data boxes in Figure 2-4 and Figure 2-5, measurements of conductivity, dissolved oxygen, ammonia, pH, and temperature indicate that exposures at these positions are not different from other chamber positions, and there are no tributaries or other obvious sources of contaminants near these chambers. Further, these position-specific mortalities do not suggest a dose-response relationship e.g., toxicity caused by ammonia or other effluent related contaminants. To date the team has no solid explanation for these mortalities, and based upon U.S. EPA relative sensitivity data, would have expected the fathead minnows at these same locations to be as susceptible as the channel catfish if the cause was ammonia. Hypotheses discussed have included bacterial or fungal disease transmitted among the catfish in the same chamber; osmotic stress; stress to channel catfish in confined spaces; or possibly some subsurface seep or release at these specific chamber positions that did not affect any other chamber positions.

Figure 2-4 Distribution of Channel Catfish and Fathead Minnow Mortality at *in situ* Location E



POSITION REMOVED AFTER 15 DAYS

CHAMBER POSITION NUMBER

0 TO 15 DAY MORTALITY

15 TO 30 DAY MORTALITY

FH- FATHEAD MINNOW

CC- CHANNEL CATFISH

WATER QUALITY PARAMETERS IN SITU LOCATION E, 8 OCTOBER 1999, 0844 HOURS.

POSTION	pH pH UNITS	CONDUCTIVITY UMHO/CM	DISSOLVED OXYGEN mg/L	TEMPERATURE DEGREES C	TOTAL NH3N mg/L
8	7.73	5060	6.36	18.7	10.31
10	7.71	4780	6.51	19.2	10.99
15	7.69	4780	7.09	19.2	11.01
16	7.72	5150	6.52	18.7	10.51
HYDROLAB	7.66	4640	6.47	19.4	11.32
19/TEST	7.67	4790	7.29	19.2	11.15
20	7.71	5260	6.46	18.5	10.47
23	7.64	4870	7.11	19	10.94
24	7.66	5210	6.11	18.3	10.41

NOT TO SCALE

FIGURE: 2-4
DISTRIBUTION OF CHANNEL CATFISH AND FATHEAD MINNOW MORTALITY AT IN SITU LOCATION. E, 21 SEPTEMBER – 21 OCTOBER 1999.

EA ENGINEERING, SCIENCE, AND TECHNOLOGY

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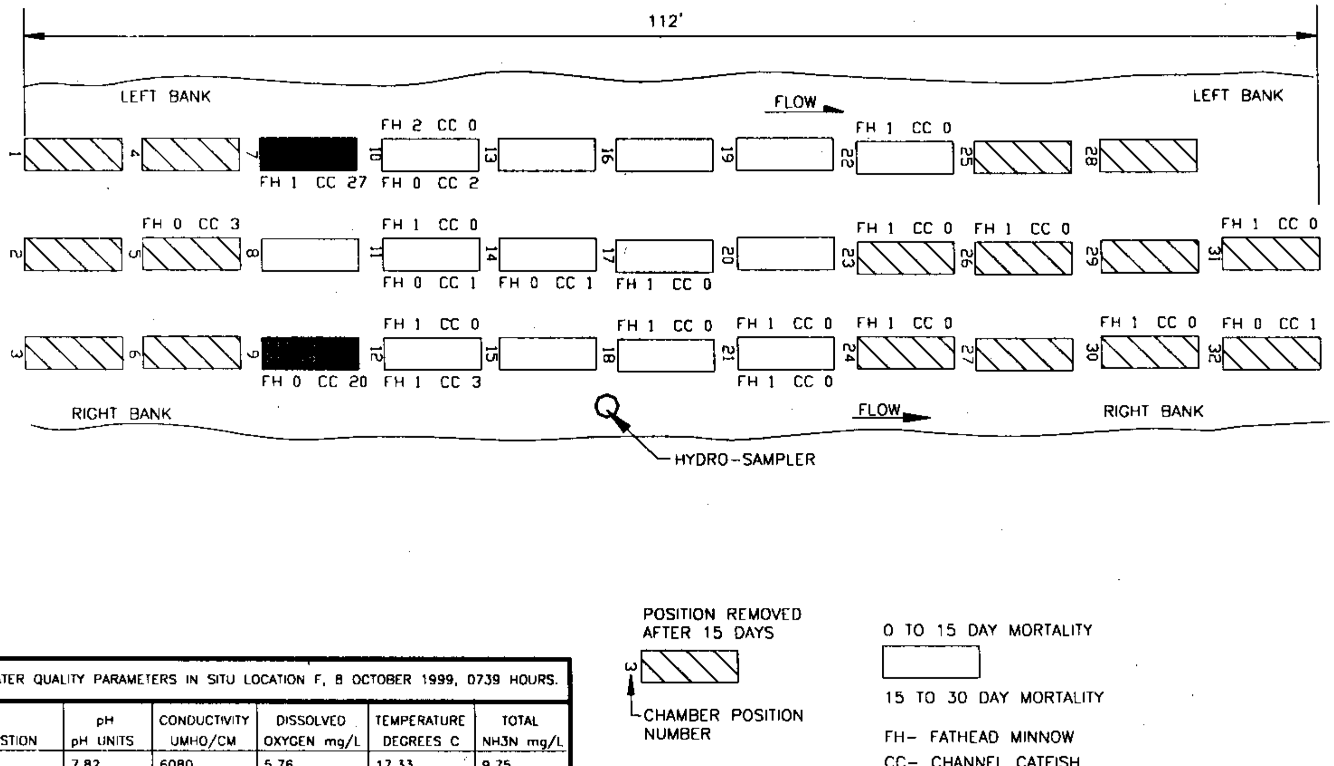
Figure 2-5 Distribution of Channel Catfish and Fathead Minnow Mortality at *in situ* Location F

SITE "F"



WATER QUALITY PARAMETERS IN SITU LOCATION F, 8 OCTOBER 1999, 0739 HOURS.

POSTION	pH pH UNITS	CONDUCTIVITY UMHO/CM	DISSOLVED OXYGEN mg/L	TEMPERATURE DEGREES C	TOTAL NH3N mg/L
7	7.82	6080	5.76	17.33	9.75
8	7.81	5840	5.71	17.5	10.16
9	7.77	5770	5.97	17.5	10.29
16	7.8	6150	5.94	17.1	9.71
17	7.8	5730	5.91	17.5	10.13
18	7.77	5710	5.89	17.6	10.15
19	7.75	6120	3.68	17.8	9.62
20	7.75	5890	5.79	17.4	10.02
21	7.74	5820	5.75	17.5	10.24
HYDRO	7.77	5770	5.97	17.5	10.42

DRAWING NAME: C:\ACAD2000\Drawing1.dwg DRAWN BY: sdolph
DATE:02/24/2000 TIME:15:53

NOT TO SCALE

FIGURE: 2-5
DISTRIBUTION OF CHANNEL CATFISH AND FATHEAD MINNOW MORTALITY AT IN SITU LOCATION F, 21 SEPTEMBER – 21 OCTOMBER 1999.

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3.0 FISH GROWTH AND BIOMASS EVALUATION

Growth and biomass[3] at each station were key chronic sublethal endpoints evaluated in the *in situ* toxicity test program. Per the work plan, the feeding of fish (and chamber maintenance) was performed two times a day for the duration of the study. No feedings were missed during the 30-day study. After carefully cleaning each chamber, the channel catfish were fed 2.5 percent of their initial body weight with sinking salmon starter chow. Fathead minnows were fed 5 percent of their initial wet body weight with Tetra Min® sized through a number 35 sieve (0.5 mm). Fish were then observed for several minutes to note feeding behavior and indications of stress or mortality. Chamber maintenance, feeding, water quality measurements, and record keeping were performed in the same sequential order on each visit. At the end of the 15- and 30-day exposure periods, the surviving fish from each chamber were preserved, returned to the laboratory and dried overnight in an oven at 100°C to obtain a dry weight measure of growth (in grams). The 15- and 30-day chamber biomass data for channel catfish and fathead minnows are presented in Figure 2-6 and Figure 2-7 and in Table 2-3A and Table 2-3B.

3.1 15-Day Exposure Results for Growth

Although U.S. EPA's (1998, 1999) ammonia criteria document discusses clear preference for chronic test exposures of 30 days or longer, and biomass rather than just growth, the 15-day growth results are briefly discussed here. Figure 2-8 and Table 2-3A summarize the growth data obtained for both species.

- For fathead minnows, there was substantial growth during the 15-day exposure period that varied between stations, but does not appear to be related to concentrations of ammonia. As shown in Figure 2-8, the highest percent growth (relative to initial weights) was at Stations G (130.7%) and D (127.6%), and at upstream station A (120.3%). The lowest percent growth was at upstream Station B (45.1%).

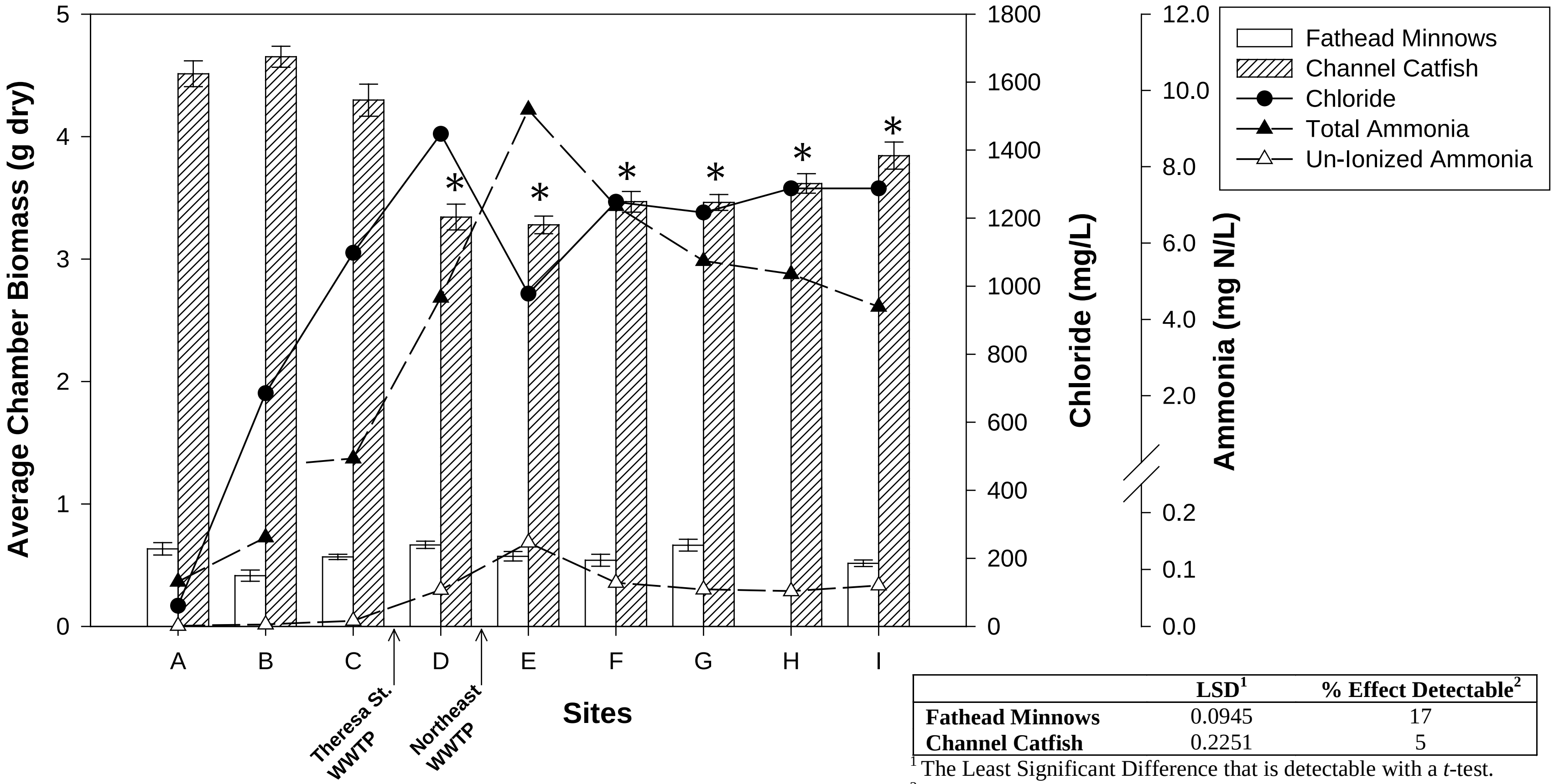
- For channel catfish, the percent growth was lower than that of the fathead minnows, and the highest relative growth occurred at the three upstream stations (Figure 2-8 and Table 2-3A). It is noteworthy that although the mean chloride concentrations at Upstream Stations A, B and C ranged from approximately 60-1,100 mg/L, the 15-day growth data for the channel catfish does not substantively differ for these three stations.

- Channel catfish growth data from the 1997 methods development research were compared to the results from the 1999 *in situ* program. As shown in Tables 2-4A and 2-4B, the 1997 methods development study started with larger catfish (0.546 g versus 0.187 g), and the percent increase was smaller in the 1997 versus the 1999 study (29 % versus 55 percent for similar exposure durations (14-15 days).

[3] Biomass is defined as the total fish biomass within a chamber; and this includes the combined effects of survival and growth within each exposure group. Biomass (rather than just growth) is the predominant chronic endpoint used for fish studies in U.S. EPA's (1998, 1999) ammonia criteria documents.

Figure 2-6 Summary of 15-Day Average Chamber Biomass

Average chamber biomass (±1 SE) after the 15-day *in situ* experiment. Asterisks (∗) indicate the stations downstream of the WWTPs that show significant impairment relative to the control Site C, as determined by pairwise one-tailed *t*-tests (H$_0$: $\overline{X}_C \le \overline{X}_i$; $\alpha = 0.05$). Average chloride and both total and un-ionized ammonia concentrations are superimposed. See Appendix 2 for additional descriptions of water chemistry.

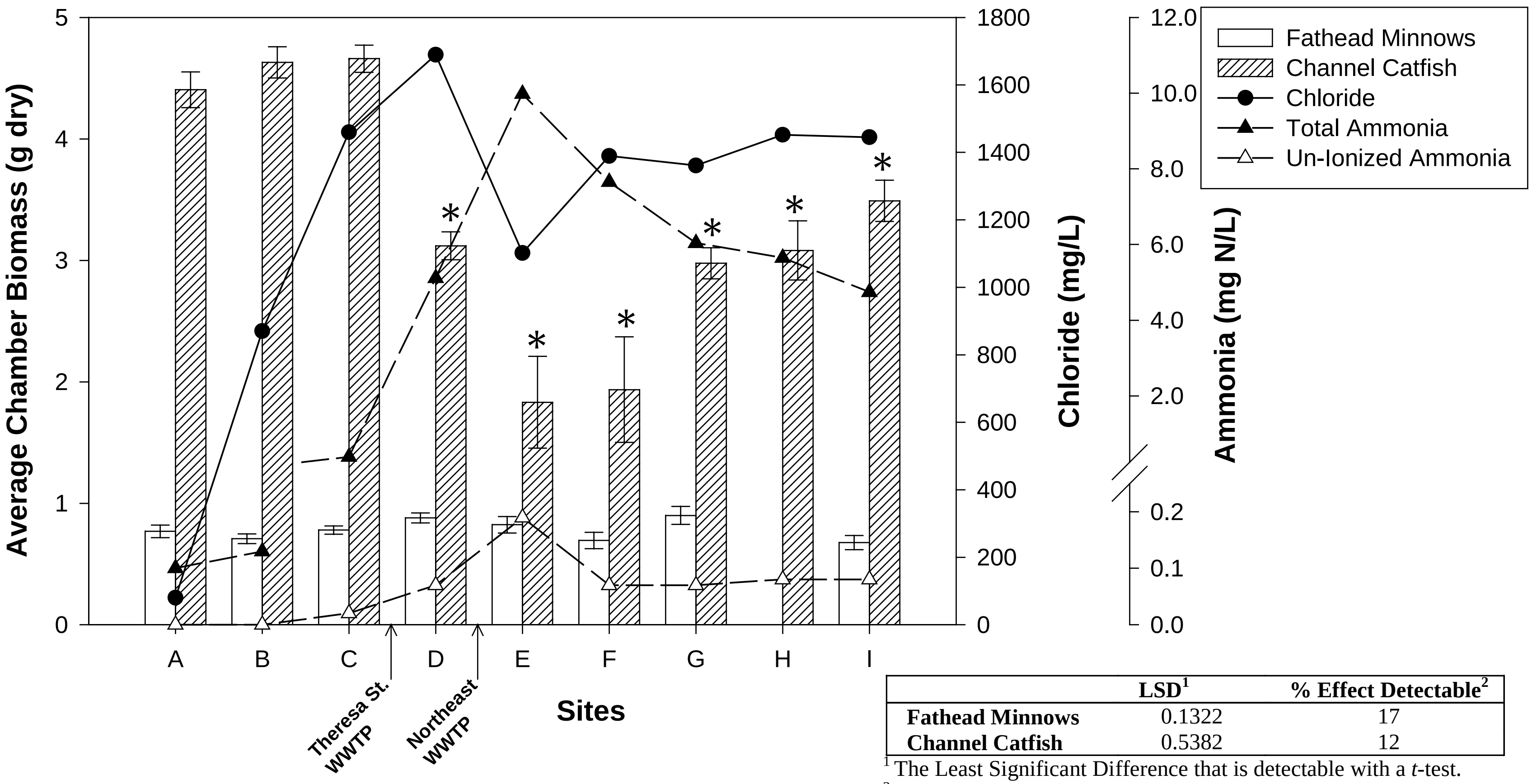


	LSD[1]	% Effect Detectable[2]
Fathead Minnows	0.0945	17
Channel Catfish	0.2251	5

[1] The Least Significant Difference that is detectable with a *t*-test.
[2] The 20% effect relative to Site C.

Figure 2-7 Summary of 30-Day Average Chamber Biomass

Average chamber biomass (±1 SE) after the 30-day *in situ* experiment. Asterisks (*) indicate the stations downstream of the WWTPs that show significant impairment relative to the control Site C, as determined by pairwise one-tailed *t*-tests (H_0: $\bar{X}_C \leq \bar{X}_i$; $\alpha = 0.05$). Average chloride and both total and un-ionized ammonia concentrations are superimposed. See Appendix 2 for additional descriptions of water chemistry.



	LSD[1]	% Effect Detectable[2]
Fathead Minnows	0.1322	17
Channel Catfish	0.5382	12

[1] The Least Significant Difference that is detectable with a *t*-test.
[2] The 20% effect relative to Site C.

Table 2–3A. The 15-Day Dry Weights and Percent Growth for Fathead Minnows and Channel Catfish

	Fathead Minnow					Channel Catfish				
Site	Dry Weight (SE)		% Growth (SE)		n	Dry Weight (SE)		% Growth (SE)		n
Initial	0.0203	(0.0017)	NA	NA	20	0.1865	(0.0083)	NA	NA	20
A	0.0448	(0.0023)	120.29	(11.46)	113	0.3008	(0.0082)	61.28	(4.42)	120
B	0.0295	(0.0013)	45.10	(6.53)	112	0.3051	(0.0085)	63.57	(4.58)	122
C	0.0394	(0.0017)	93.95	(8.14)	115	0.2889	(0.0078)	54.88	(4.16)	119
D	0.0463	(0.0018)	127.62	(8.98)	115	0.2228	(0.0065)	19.47	(3.49)	120
E	0.0398	(0.0022)	95.67	(10.66)	115	0.2204	(0.0068)	18.17	(3.66)	119
F	0.0396	(0.0021)	94.52	(10.38)	109	0.2391	(0.0066)	28.21	(3.52)	116
G	0.0469	(0.0020)	130.74	(9.93)	113	0.2308	(0.0067)	23.72	(3.62)	120
H	-	-	-	-	-	0.2432	(0.0068)	30.36	(3.66)	119
I	0.0371	(0.0015)	82.54	(7.41)	111	0.2584	(0.0067)	38.55	(3.58)	119

SE = standard error of the mean

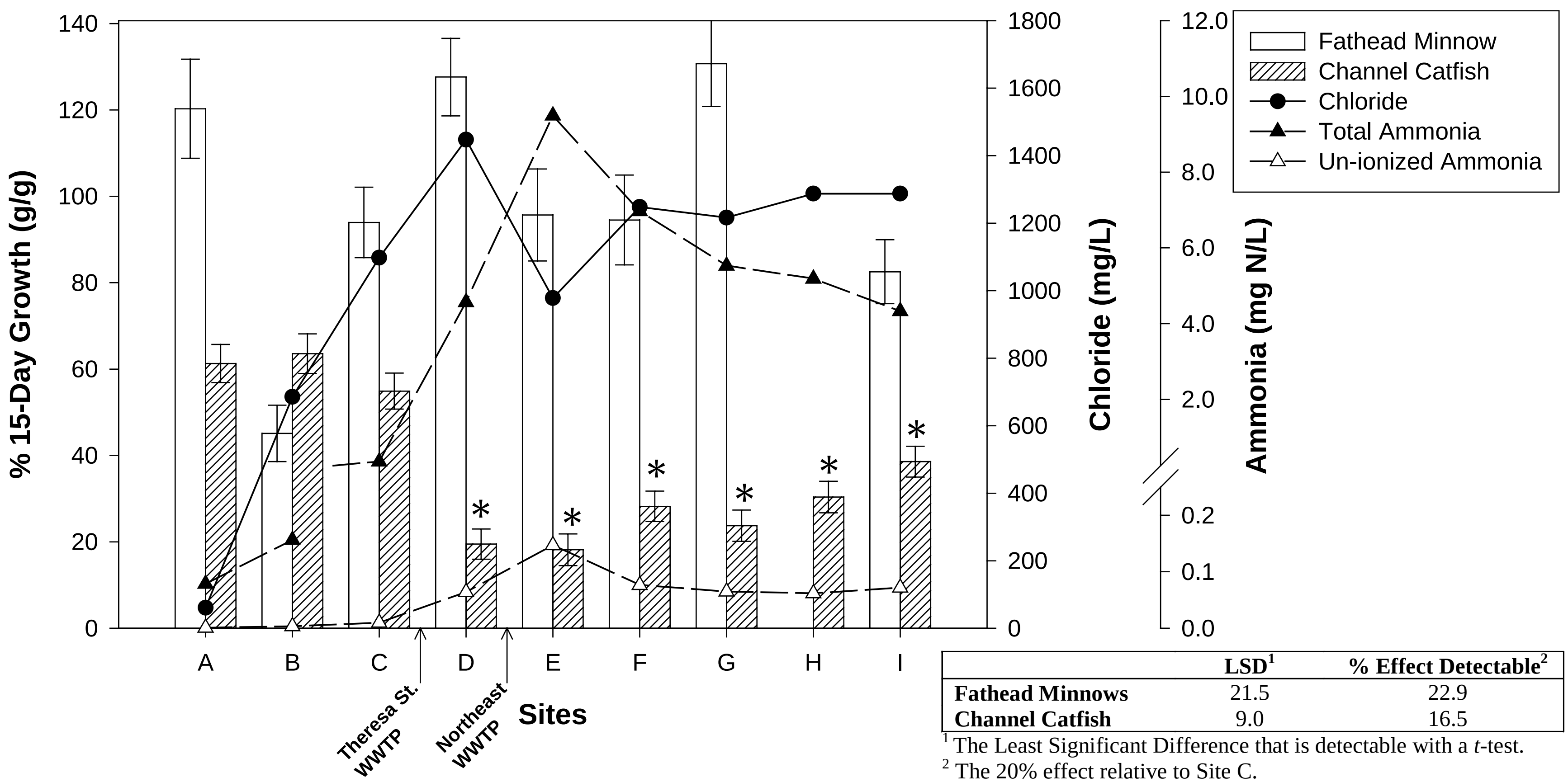
Table 2–3B. The 30-Day Dry Weights and Percent Growth for Fathead Minnows and Channel Catfish

	Fathead Minnow					Channel Catfish				
Site	Dry Weight (SE)		% Growth (SE)		n	Dry Weight (SE)		% Growth (SE)		n
Initial	0.0203	(0.0017)	NA	NA	20	0.1865	(0.0083)	NA	NA	20
A	0.0575	(0.0042)	182.66	(20.50)	107	0.2937	(0.0078)	57.44	(4.19)	120
B	0.0534	(0.0045)	162.65	(21.93)	106	0.3166	(0.0093)	69.73	(4.99)	117
C	0.0542	(0.0020)	166.53	(9.82)	115	0.3056	(0.0075)	63.83	(4.01)	122
D	0.0617	(0.0024)	203.44	(11.69)	114	0.2115	(0.0065)	13.40	(3.47)	118
E	0.0604	(0.0029)	197.01	(14.12)	109	0.1885	(0.0080)	1.05	(4.27)	68
F	0.0518	(0.0023)	154.94	(11.21)	107	0.2052	(0.0083)	10.03	(4.47)	66
G	0.0643	(0.0034)	215.99	(16.64)	112	0.2107	(0.0059)	12.95	(3.17)	113
H	-	-	-	-	-	0.2201	(0.0062)	17.99	(3.34)	112
I	0.0515	(0.0022)	153.43	(10.63)	105	0.2386	(0.0067)	27.92	(3.62)	117

SE = standard error of the mean

Figure 2-8 Summary of the 15-Day Fish Percent Growth

Mean dry weights (±1 SE) after the 15-day *in situ* experiment. Asterisks (∗) indicate the stations downstream of the WWTPs that show significant impairment relative to the control Site C, as determined by pairwise one-tailed *t*-tests (H_0: $\overline{X}_C \leq \overline{X}_i$; $\alpha = 0.05$). The average chloride and both total and un-ionized ammonia concentrations are superimposed. See Appendix 2 for additional descriptions of water chemistry.



	LSD[1]	% Effect Detectable[2]
Fathead Minnows	21.5	22.9
Channel Catfish	9.0	16.5

[1] The Least Significant Difference that is detectable with a *t*-test.
[2] The 20% effect relative to Site C.

Table 2–4A. Baseline Dry Weights of Channel Catfish used in the 1997 and 1999 Salt Creek *in situ* Studies.

Study	N	Dry Weights (g)				Coefficient of Variation
		Average	Min	Max	STD	
1997	17	0.5462	0.4212	0.6808	0.0864	16
1999	20	0.1865	0.1210	0.2581	0.0372	20

Table 2–4B. Endpoint Dry Weights of Channel Catfish used in the 1997 and 1999 Salt Creek *in situ* Studies for Site C.

Study	Exposure	n	Dry Weights (g)				Percent Increase	Coefficient of Variation
			Average	Min	Max	STD		
1997	14 days	50	0.7065	0.4816	0.9441	0.1249	29	17
1999	15 days	119	0.2889	0.1149	0.5501	0.0846	55	29
1999	30 days	122	0.3056	0.1376	0.5052	0.0826	64	27

3.2 30-Day Exposure Results for Growth

Figure 2-9 and Table 2-3B summarize the 30-day exposure growth data for both species.

- For fathead minnows, there was substantial growth during the total 30-day exposure period—as well as between 15 and 30 days. As shown in Figure 2-9, the highest percent growth (relative to initial weights) over the 30 days was at downstream Stations G (216%), D (203.4%), and E (197.0%), and at upstream Station A (182.7%). The lowest percent growth was at the furthest downstream Station I (153.4%). Based on these 30-day exposure data, the growth of fathead minnows varied between stations but does not appear to be related to concentrations of ammonia. It is noteworthy that although the mean chloride concentrations at Upstream Stations A, B and C ranged from approximately 80-1,460 mg/L, the 30-day growth data for the fathead minnows do not substantively differ for these three stations.

- For channel catfish, the percent growth was lower than for the fathead minnows, and little additional growth occurred during the 15- to 30-day timeframe (Tables 2-3A and 2-3B). The highest relative growth occurred at the three upstream stations with percent growth values of 57.4%, 69.7%, and 63.8% at Stations A, B and C, respectively (Figure 2-9 and Tables 2-3A and 2-3B). Further, as shown in Figure 2-7, growth at Stations D through I was significantly lower than at upstream stations A, B and C. As in the 15-day catfish data, although the mean chloride concentrations at Upstream stations A, B and C ranged from approximately 80-1,460 mg/L, the 30-day growth data for the channel catfish do not substantively differ for these three upstream stations.

3.3 Discussion of Ambient Temperature During the In situ Study

Ambient Salt Creek temperature data for the 30-day (21 September to 20 October) *in situ* study are presented in Figure 2-10 and Figure 2-11. Because Salt Creek is quite shallow in lower flow periods of the year, diurnal temperature changes can be substantial. These ambient temperature data show several points:

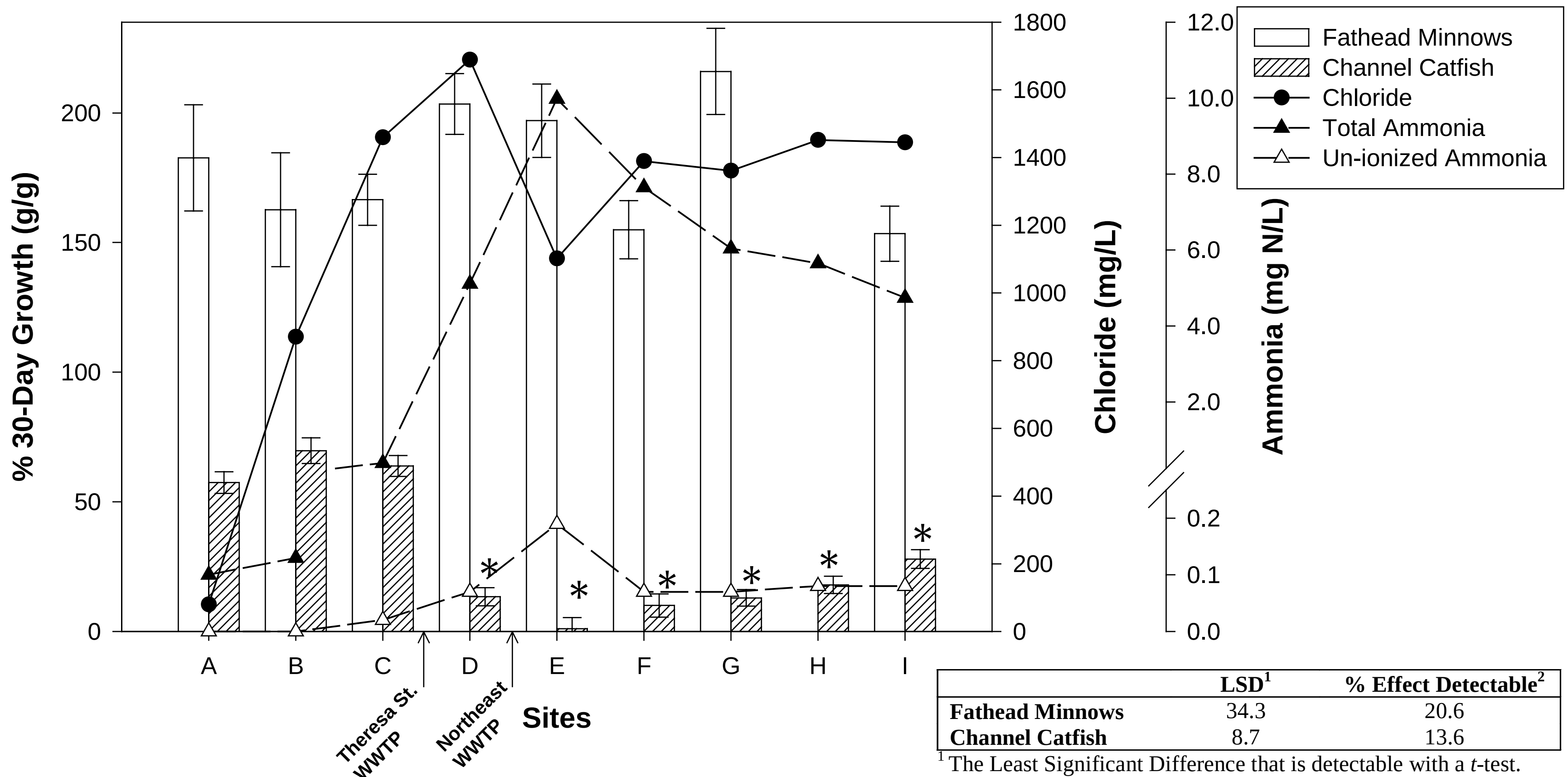
- The introduction of municipal wastewaters from the Theresa Street and Northeast WWTPs increase the temperature of Salt Creek during cooler periods of the year. As shown in Figure 11, the increase from Theresa was approximately 1.5°C, and the increase from the combined Theresa Street and Northeast WWTP discharges was approximately 3°C based upon the 30-day average values. This ambient temperature increase continues downstream but is dampened with distance. It should be noted that during the warm weather months of the year, the effluents from these facilities are expected to slightly *cool* the downstream ambient temperatures.

- Ambient temperature has been shown to affect the growth of aquatic fish and invertebrates. As discussed later in this report, we have evaluated the fish growth and biomass data with and without temperature correction.

- The 1997 method development studies in Salt Creek occurred during the same period in early October of time as the 1999 *in situ* study (Figure 2-10).

Figure 2-9 Summary of the 30-Day Fish Percent Growth

Mean dry weights (±1 SE) after the 30-day *in situ* experiment. Asterisks (∗) indicate the stations downstream of the WWTPs that show significant impairment relative to the control Site C, as determined by pairwise one-tailed *t*-tests (H_0: $\bar{X}_C \le \bar{X}_i$; $\alpha = 0.05$). The average chloride and both total and un-ionized ammonia concentrations are superimposed. See Appendix 2 for additional descriptions of water chemistry.



	LSD[1]	% Effect Detectable[2]
Fathead Minnows	34.3	20.6
Channel Catfish	8.7	13.6

[1] The Least Significant Difference that is detectable with a *t*-test.
[2] The 20% effect relative to Site C.

Figure 2-10 1997 and 1999 Temperature Recordings at *in situ* Location C

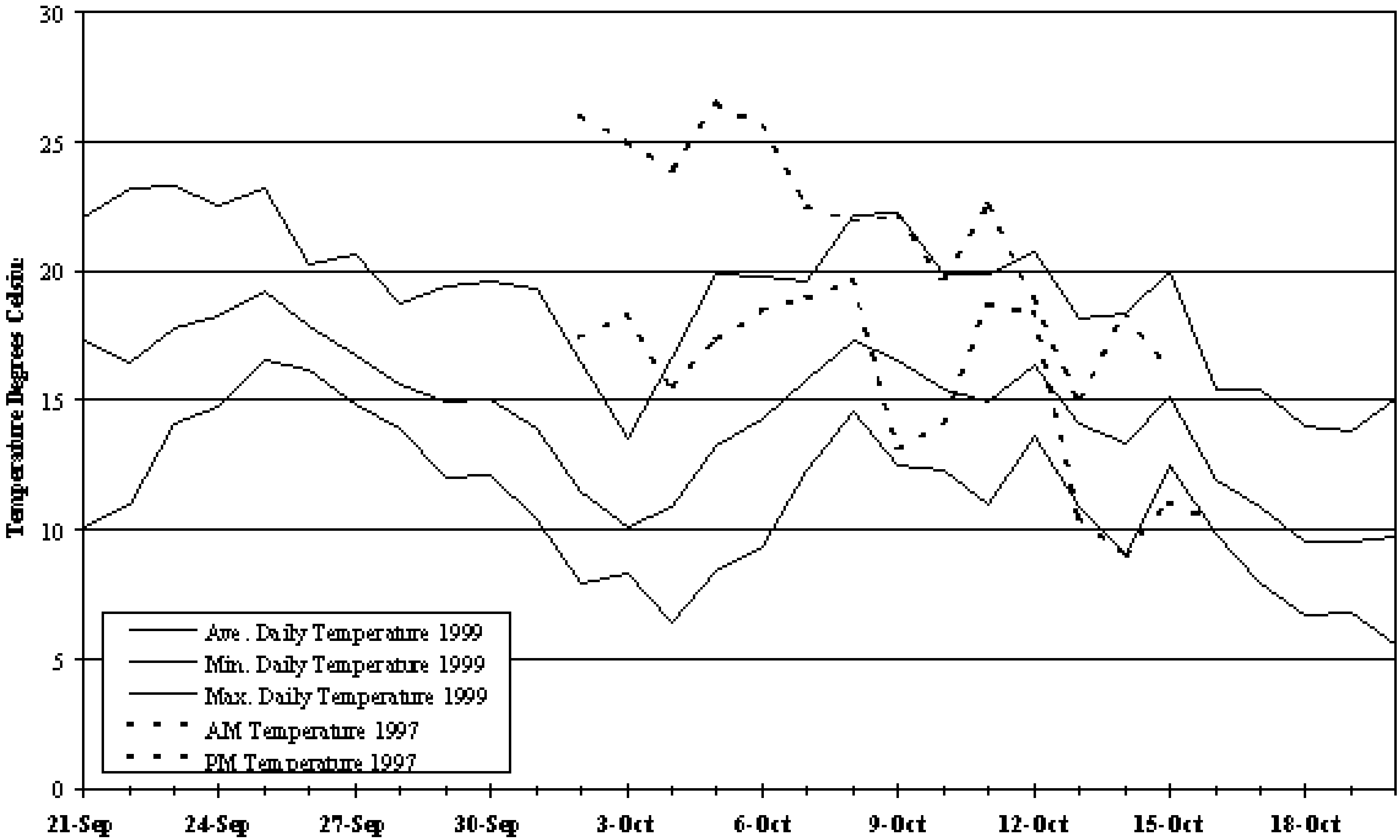
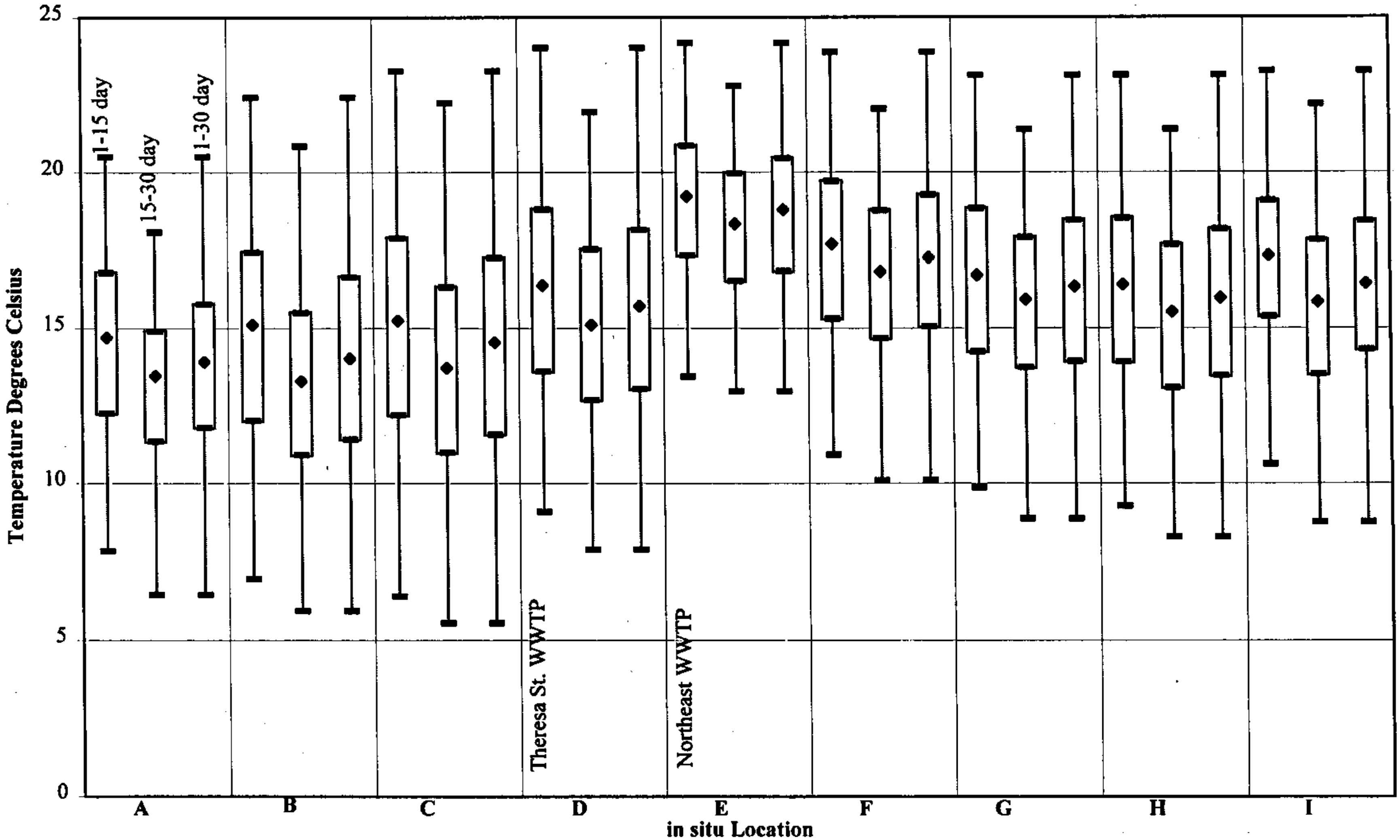


Figure 2-11 1-15 Day, 15-30 Day, and 1-30 Day Maximum, 75th Percentile, Average 25th Percentile, and Minimum Temperatures During the Salt Creek *in situ* Study



4.0 RESULTS FROM THE *IN SITU* TESTING

The goal of the *in situ* program was to calculate 30-day IC20 values for fathead minnows and channel catfish, and use those data in combination with the other studies to determine a weight-of-evidence based site-specific water quality criterion for ammonia. Note that U.S. EPA's (1999) Ammonia Criteria Updates present guidance on the preferred data. More specifically:

- 30-day exposures are close to the minimum test duration used for chronic fish in the ammonia criteria update. In Table 5 of U.S. EPA's Ammonia Update, 70 percent (14/20) of the chronic fish tests had exposure durations of between 28 and 32 days. Two of the remaining 6 tests were life cycle tests and the other four were 42-73 day rainbow trout tests.

- Biomass is the preferred endpoint (versus growth) because it combines the effects of survival and growth for each exposure concentration, and "*because growth effects on juvenile fish are sometimes transient, so that short chronic tests with such tests might overestimate risk*" (p. 71) For this specific reason, U.S. EPA (1999) chose to <u>not</u> use growth effect data for channel catfish for criteria derivation (p. 71).

The calculated *in situ* IC20 values for total ammonia and un-ionized ammonia are presented in Tables 2-5A and 2-5B below, respectively. It is important to recognize that these IC20 values reflect the actual water quality conditions of Salt Creek during the 30-day exposure period (e.g., the in-stream variability of ammonia, pH, temperature, chloride and other background water quality characteristics).

Table 2–5A. Calculated IC_{20} Values for Total Ammonia.
Standard errors for the IC20 were computed from the bootstrap method with 1,000 resamples.

	IC20 ± SE (mg N/L)			
	Fathead Minnows		Channel Catfish	
	15-Day	30-Day	15-Day	30-Day
% Mortality	na	na	na	6.75 ± 0.37
% Growth	na	na	3.02 ± 0.68	1.94 ± 0.29
Total Biomass	> 9.5	> 9.98	7.33 ± 1.61	**3.85 ± 0.48**

Table 2–5B. Calculated IC_{20} values for Un-ionized Ammonia.
Standard errors for the IC20 were computed from the bootstrap method with 1,000 resamples.

	IC20 ± SE (mg N/L)			
	Fathead Minnows		Channel Catfish	
	15-Day	30-Day	15-Day	30-Day
% Mortality	na	na	na	0.10 ± 0.02
% Growth	na	na	0.03 ± 0.00	0.03 ± 0.00
Total Biomass	na	na	0.09 ± 0.02	0.05 ± 0.00

4.1 Channel Catfish Results

The results of the 15- and 30-day in-situ exposures are summarized in Figure 2-12 and Figure 2-13, respectively. The 30-day IC20 value for the channel catfish was calculated to be 3.85 mg N/L based on total biomass (Figure 2-13). The corresponding 15-day IC20 value was calculated as 7.33 mg N/L (Figure 2-12), which is within the relative range expected for a shorter duration test. Note that these channel catfish data were not "adjusted" in any way to address the unexplained mortalities at Stations E and F (see Section 2.2). The calculated IC20 is not affected by the inclusion of the observed mass mortalities, since they occurred only at Stations E and F, and (as shown in Figure 2-13) the IC20 value is calculated based on the data from Stations C, I, H, and G.

4.2 Fathead Minnow Results

Thirty day IC20 values for the fathead minnow are >9.98 mg N/L, because there was less than a 20 percent decrease in biomass at downstream stations compared to the control (Station C) at the highest 30-day average in-stream exposure concentration. The lack of a dose-response to the fathead minnows is clearly shown in Figure 2-12 and Figure 2-13. Fathead minnows showed significant growth in both the 15- and 30-day exposure periods (see Section 3.2). This was unexpected given the literature values presented in the U.S. EPA's (1998) ammonia criteria update, which were used for the design of the *in situ* program. One explanation for the fathead minnow result identified in EPA's recently released 1999 Update is that juvenile fathead minnows have been shown to be less sensitive than early life stages by a factor of approximately 3 (EPA 1999, p. 69-70). Conversely, laboratory data on juvenile channel catfish "*seem to be as, or more, sensitive than the available early life stage tests*" (p.71).

Figure 2-12 The 15-Day Total Chamber Biomass versus Mean Total Ammonia Concentration

Dose-response relationship between total chamber biomass and mean total ammonia concentration during the 15-day *in situ* experiment. The mean total chamber biomass (solid line) is used to calculate the IC_{20}. The dashed line indicates the IC_{20} for channel catfish. For fathead minnows, a 20% effect relative to the reference site C was not observed.

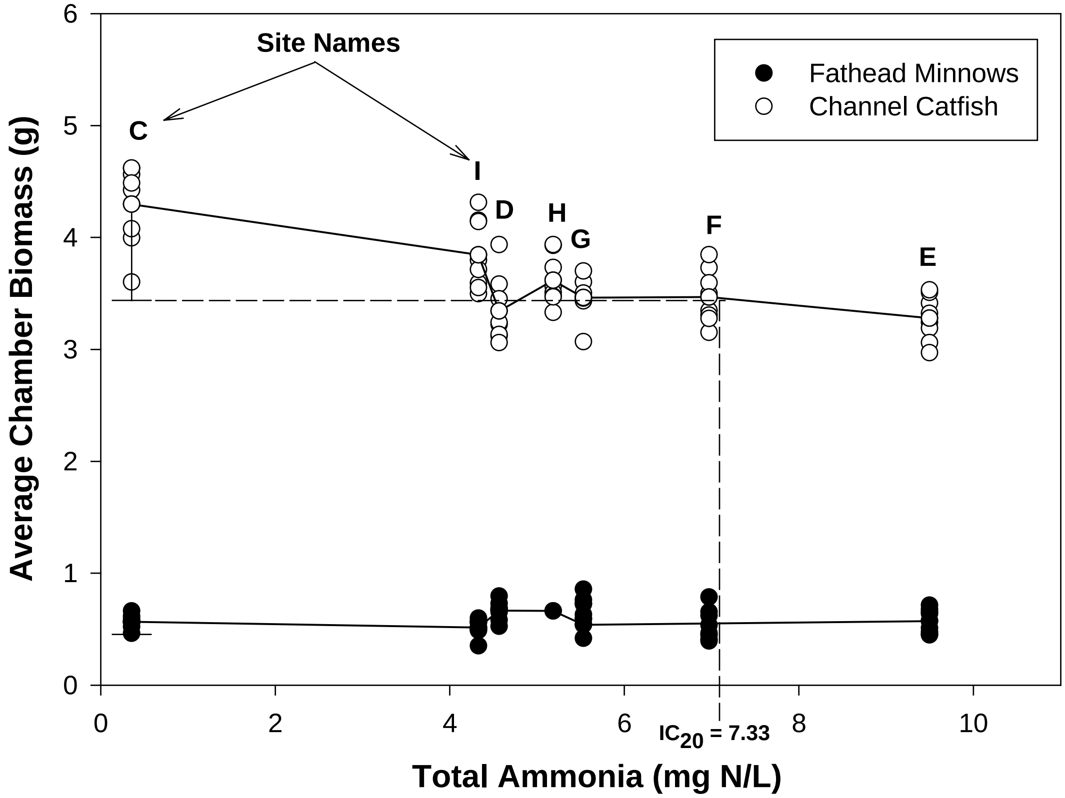
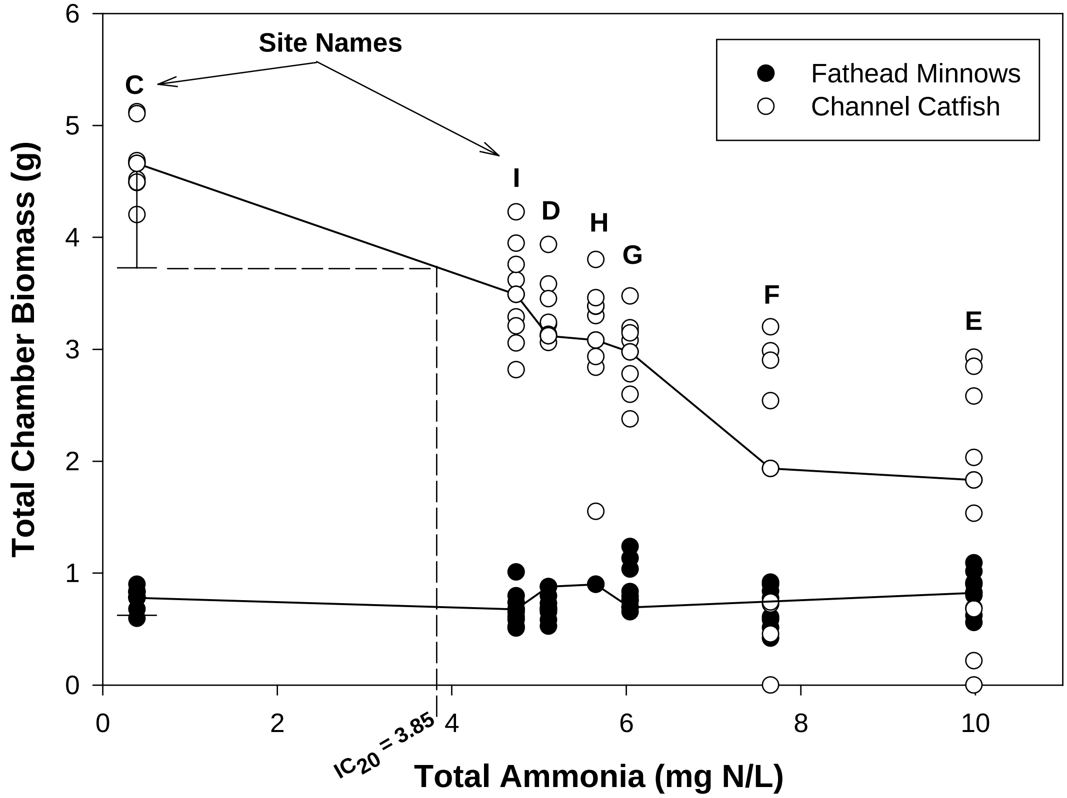


Figure 2-13 The 30-Day Total Chamber Biomass versus Mean Total Ammonia Concentration

Dose-response relationship between total chamber biomass and mean total ammonia concentration during the 30-day *in situ* experiment. The mean total chamber biomass (solid line) is used to calculate the IC_{20}. The dashed line indicates the IC_{20} for channel catfish. For fathead minnows, a 20% effect relative to the reference site C was not observed.



4.3 Statistical Associations Between Water Quality Variables and Biological Endpoints

Pearson product-moment correlation coefficients (r) were computed to obtain the degree of statistical association between biological endpoints and selected water quality parameters from the *in situ* program. The matrix of correlation coefficients for the 30-day channel catfish results (Table 2-6A and Table 2-6B below) shows a high degree of inverse association between total biomass and total ammonia ($r = -0.96$, $p < 0.001$). There were no significant correlations observed in the 30-day fathead minnow data.

Table 2–6a. Correlation Coefficients between Water Quality Parameters and Toxicity Metrics for the 30-Day Channel Catfish
Pearson Product-Moment Correlation Coefficients and their probabilities of significance for the H_0: $r = 0$

	Total Ammonia	Un-ionized Ammonia	Conductivity	Chlorides	Temperature	pH
Dry Weight	0.32 (0.4460)	0.33 (0.4127)	-0.04 (0.9335)	0.04 (0.9224)	0.26 (0.5262)	-0.15 (0.7165)
%Mortality	0.18 (0.6625)	0.12 (0.8096)	-0.70 (0.0546)	-0.72 (0.0439)	0.19 (0.6479)	-0.56 (0.1484)
%Growth	0.32 (0.4460)	0.33 (0.4127)	-0.04 (0.9335)	0.04 (0.9224)	0.26 (0.5262)	-0.15 (0.7165)
Total Biomass	0.25 (0.5517)	0.26 (0.5111)	0.11 (0.8037)	0.19 (0.6465)	0.21 (0.6195)	-0.04 (0.9333)

Table 2–6b. Correlation Coefficients between Water Quality Parameters and Toxicity Metrics for the 30-Day Fathead Minnows
Pearson Product-Moment Correlation Coefficients and their probabilities of significance for the H_0: $r = 0$.

	Total Ammonia	Un-ionized Ammonia	Conductivity	Chlorides	Temperature	pH
Dry Weight	-0.96 (0.0000)	-0.84 (0.0055)	-0.50 (0.1719)	-0.50 (0.1694)	-0.89 (0.0013)	0.68 (0.0441)
%Mortality	0.68 (0.0449)	0.58 (0.1116)	0.08 (0.8348)	0.07 (0.8628)	0.75 (0.0190)	-0.60 (0.0892)
%Growth	-0.96 (0.0000)	-0.84 (0.0055)	-0.50 (0.1719)	-0.50 (0.1694)	-0.89 (0.0013)	0.68 (0.0441)
Total Biomass	-0.97 (0.0000)	-0.85 (0.0050)	-0.37 (0.3271)	-0.36 (0.3369)	-0.96 (0.0001)	0.74 (0.0213)

5.0 SUMMARY AND CONCLUSIONS

An *in situ* toxicity test was performed in which caged fish were continuously exposed for 30-days at nine in-stream locations in Salt Creek. Two fish species were selected for this program: fathead minnows (*Pimephales promelas*) and channel catfish (*Ictalurus punctatus*). This selection was based on their relative sensitivity to ammonia, the availability of experimental protocols, the ability to obtain sufficient numbers of experimental organisms, and their importance in the Salt Creek ecosystem.

The selection of exposure locations was based on two factors: in-stream concentrations of total ammonia; and natural concentrations of salinity. Five locations downstream of the Northeast WWTP were used (Stations E, F, G, H, and I), in addition to one ammonia "control" station immediately upstream from the Northeast WWTP (Station D). To evaluate the effect of salinity, three additional upstream salinity "controls" were located above the Theresa Street WWTP, to reflect the potential range of salinities at the downstream stations (Stations A, B, and C). This experimental design resulted in a total of nine stations. Extensive water quality monitoring was conducted at each in-stream station throughout the experiment, as well as effluent monitoring at both WWTPs.

The experimental design evaluated two toxicological endpoints: mortality and growth. Each is discussed below.

- Mean mortality over the 30-day exposure for the fathead minnows averaged 6.8 percent for the eight stations, with the highest mortalities at upstream stations A and B, and downstream stations E and F. The channel catfish data are more difficult to explain. There was almost no mortality at any of the sites except at Stations E and F, which are the first two sites downstream of the Northeast WWTP outfall. At those two stations total mortality was 42.0 and 44.8 percent respectively, with the majority of the mortality occurring at only a few of the chamber positions. It is noteworthy that chambers immediately in front of and behind these positions exhibited almost no mortality, and fathead minnows located in these same two positions showed almost no mortality, leading to the conclusion that the mortalities do <u>not</u> appear to be related to ammonia exposure.

- Growth and biomass[4] at each station were key chronic sublethal endpoints evaluated in the *in situ* toxicity test program. The highest percent growth (relative to initial weights) over the 30 days was at downstream Stations G (216%), D (203.4%), and E (197.0%), and at upstream Station A (182.7%). The lowest percent growth was at the furthest downstream Station I (153.4%). Based on these 30-day exposure data, the growth of fathead minnows varied between stations but does *not* appear to be related to concentrations of ammonia. For channel catfish, the percent growth was lower than for the fathead minnows. The highest relative growth occurred at the three upstream stations with percent growth values of 57.4, 69.7 and 63.8 % at Stations A,

[4] Biomass is defined as the total fish biomass within a chamber; and this includes the combined effects of survival and growth within each exposure group. Biomass (rather than just growth) is the predominant chronic endpoint used for fish studies in U.S. EPA's (1998, 1999) ammonia criteria documents.

B and C, respectively. Growth at downstream Stations D through I was significantly lower than at the upstream stations.

Pearson Product-Moment correlation coefficients (r) were computed to determine the degree of statistical association between biological endpoints and selected water quality parameters from the *in situ* program. The matrix of correlation coefficients for the 30-day channel catfish results shows a high degree of inverse association between total biomass and total ammonia ($r = -0.96$, $p<0.001$). There were, however, *no* significant correlations observed in the 30-day fathead minnow data.

The scientific goal of the *in situ* program was to calculate 30-day IC20 values for fathead minnows and channel catfish, and use those data in combination with the other studies to determine a weight-of-evidence based site-specific water quality criterion for ammonia. It is important to recognize that the calculated IC20 values reflect the actual water quality conditions of Salt Creek during the 30-day exposure period (e.g., the in-stream variability of ammonia, pH, temperature, chloride and other background water quality characteristics).

- The 30-day IC20 value for the channel catfish was calculated to be 3.85 mg N/L based on total biomass.
- The thirty day IC20 values for the fathead minnow are >9.98 mg N/L, because there was less than a 20 percent decrease in biomass at downstream stations compared to the control (Station C) at the highest 30-day average in-stream exposure concentration.

These *in situ* IC20 values can be used with the other site-specific data using a weight of evidence approach to determine a numeric site-specific water quality standard which will be fully protective of Salt Creek.

6.0 LITERATURE CITED

U.S. Environmental Protection Agency. 1998. Update of Ambient Water Quality Criteria for Ammonia. Document # EPA 822-R-98-008. Office of Water, Washington D.C.

U.S. Environmental Protection Agency. 1999. 1999 Update of Ambient Water Quality Criteria for Ammonia. Document # EPA 822-R-99-014. Office of Water, Washington D.C. Issued 22 December 99.

APPENDIX 2-1: Summary Statistics of the 15-Day Water Chemistry Data

		Sites								
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Temperature, °C	**N**	362	362	361	357	358	358	338	340	320
	Mean	14.48	14.71	15.11	16.33	19.11	17.61	16.68	16.37	16.23
	Median	14.71	15.11	15.30	16.37	19.22	17.73	16.70	16.41	16.25
	SD	2.89	3.51	3.95	3.51	2.43	2.94	3.03	3.16	3.31
	25% Quantile	12.27	12.03	12.23	13.61	17.38	15.31	14.25	13.96	13.69
	75% Quantile	16.80	17.44	17.90	18.81	20.86	19.72	18.84	18.52	18.66
pH, s.u.	**N**	362	362	361	357	358	358	339	340	320
	Mean	7.73	7.76	7.97	7.68	7.61	7.52	7.57	7.58	7.73
	Median	7.71	7.73	7.95	7.65	7.60	7.52	7.57	7.59	7.71
	SD	0.11	0.14	0.15	0.11	0.12	0.05	0.06	0.11	0.08
	25% Quantile	7.64	7.65	7.85	7.60	7.51	7.48	7.53	7.52	7.68
	75% Quantile	7.81	7.86	8.03	7.72	7.69	7.56	7.59	7.66	7.75
Cl, mg/L	**N**	31	31	31	84	90	90	89	90	90
	Mean	60	685	1098	1448	978	1248	1217	1288	1288
	Median	56	537	690	1287	964	1212	1160	1270	1224
	SD	27	324	628	366	218	338	249	274	261
	25% Quantile	35	445	569	1170	805	1000	1025	1065	1075
	75% Quantile	80	1075	1742	1784	1155	1475	1435	1515	1515
Total Ammonia	**N**	29	30	30	84	90	90	90	90	90
mg N/L	**Mean**	0.08	0.16	0.35	4.57	9.50	6.97	5.53	5.18	4.33
	Median	0.09	0.17	0.23	4.38	9.46	6.83	5.33	5.10	4.08
	SD	0.06	0.05	0.28	1.75	1.49	1.57	1.37	1.47	1.70
	25% Quantile	0.01	0.14	0.14	3.32	8.50	6.12	4.56	4.22	3.15
	75% Quantile	0.11	0.19	0.66	5.63	10.62	8.13	6.69	6.20	5.45
Unionized Ammonia,	**N**	29	30	30	83	90	90	86	87	82
mg N/L	**Mean**	0.00	0.00	0.01	0.06	0.15	0.08	0.06	0.06	0.07
	Median	0.00	0.00	0.01	0.05	0.14	0.07	0.06	0.06	0.06
	SD	0.00	0.00	0.01	0.03	0.05	0.02	0.02	0.02	0.04
	25% Quantile	0.00	0.00	0.00	0.04	0.10	0.06	0.05	0.04	0.05
	75% Quantile	0.00	0.00	0.01	0.08	0.17	0.09	0.07	0.08	0.08

APPENDIX 2-2: Summary Statistics of the Water Chemistry Data from Day 16 to 30

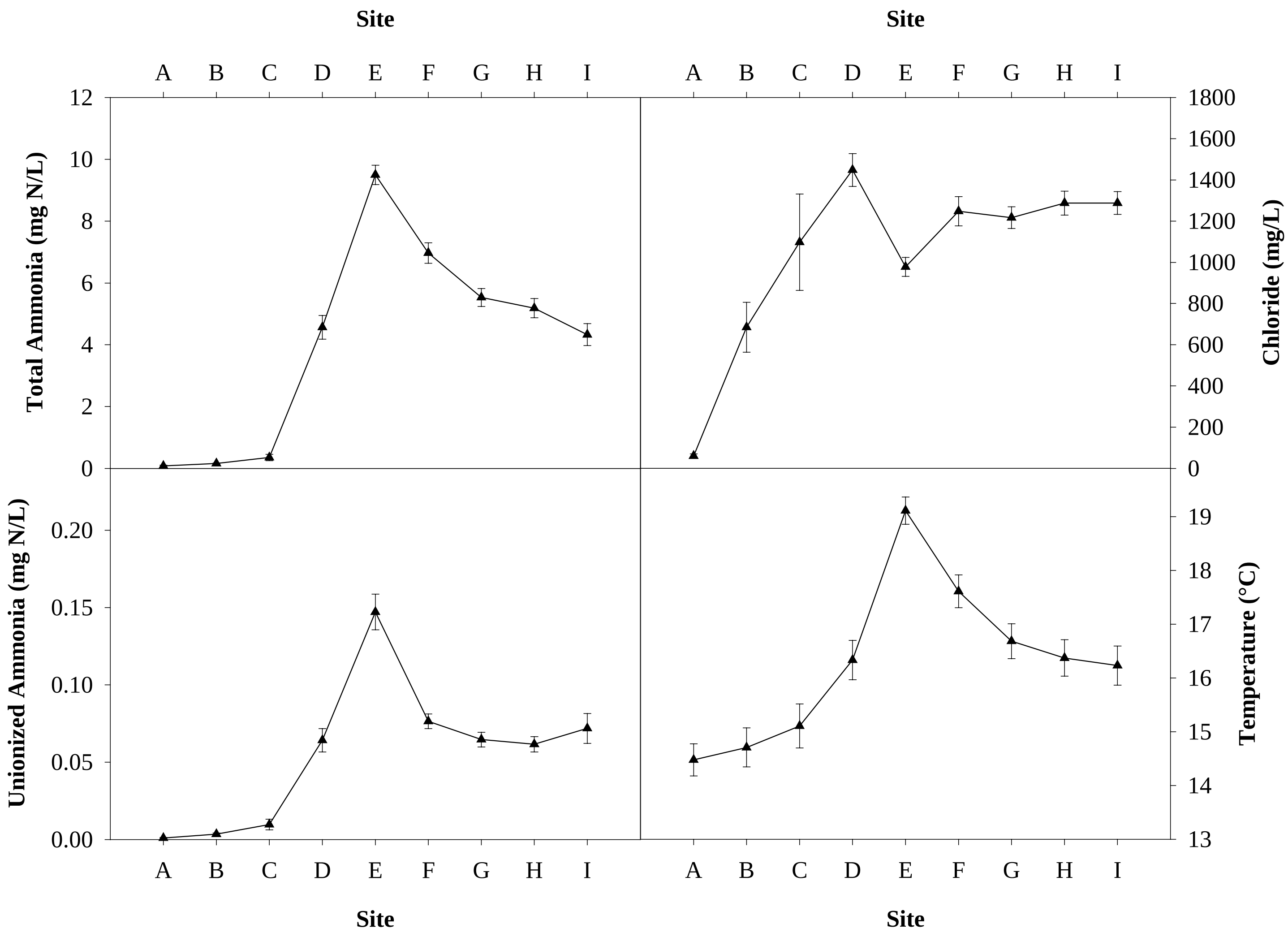
		Sites								
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Temperature, °C	**N**	358	358	358	360	360	360	360	359	359
	Mean	12.97	13.22	13.63	15.01	18.20	16.64	15.72	15.29	15.24
	Median	13.44	13.26	13.68	15.08	18.30	16.73	15.89	15.50	15.51
	SD	2.61	3.35	3.81	3.24	2.28	2.72	2.88	3.01	3.21
	25% Quantile	11.20	10.86	10.99	12.64	16.51	14.65	13.60	13.02	12.88
	75% Quantile	14.90	15.50	16.32	17.53	19.97	18.77	17.91	17.68	17.68
pH, s.u.	**N**	358	358	358	360	360	360	360	359	359
	Mean	7.72	7.85	7.98	7.71	7.81	7.42	7.61	7.74	7.80
	Median	7.69	7.76	7.92	7.72	7.80	7.45	7.61	7.75	7.77
	SD	0.11	0.25	0.21	0.07	0.07	0.17	0.05	0.11	0.08
	25% Quantile	7.64	7.64	7.79	7.65	7.75	7.43	7.57	7.68	7.73
	75% Quantile	7.82	8.10	8.18	7.77	7.86	7.48	7.64	7.81	7.86
Cl, mg/L	**N**	29	29	29	88	90	90	80	88	90
	Mean	101	1069	1847	1919	1225	1531	1522	1621	1602
	Median	97	1064	1898	1910	1220	1530	1519	1609	1604
	SD	37	109	212	162	97	111	118	123	127
	25% Quantile	80	1000	1670	1795	1150	1446	1440	1532	1507
	75% Quantile	108	1139	2016	1998	1293	1613	1598	1703	1668
Total Ammonia	**N**	29	29	29	88	90	90	80	89	90
mg N/L	**Mean**	0.12	0.11	0.43	5.62	10.47	8.33	6.62	6.12	5.15
	Median	0.10	0.10	0.45	5.26	10.55	8.35	6.50	5.77	5.00
	SD	0.05	0.02	0.18	1.67	1.29	1.31	1.29	1.43	1.35
	25% Quantile	0.10	0.10	0.34	4.55	9.48	7.45	5.62	5.21	4.19
	75% Quantile	0.10	0.12	0.56	6.62	11.42	9.20	7.29	7.12	6.19
Unionized Ammonia,	**N**	29	29	29	88	90	90	80	89	90
mg N/L	**Mean**	0.00	0.00	0.02	0.08	0.23	0.07	0.08	0.10	0.09
	Median	0.00	0.00	0.01	0.08	0.23	0.07	0.08	0.10	0.08
	SD	0.00	0.00	0.02	0.03	0.04	0.02	0.02	0.03	0.04
	25% Quantile	0.00	0.00	0.01	0.06	0.20	0.06	0.06	0.07	0.06
	75% Quantile	0.00	0.01	0.03	0.10	0.26	0.09	0.09	0.12	0.11

APPENDIX 2-3: Summary Statistics of the 30-Day Water Chemistry Data

		Sites								
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Temperature, °C	**N**	720	720	719	717	718	718	698	699	679
	Mean	13.73	13.97	14.37	15.67	18.65	17.12	16.18	15.82	15.71
	Median	13.90	14.01	14.53	15.70	18.78	17.24	16.32	15.96	15.91
	SD	2.85	3.51	3.95	3.44	2.40	2.87	2.99	3.13	3.29
	25% Quantile	11.79	11.41	11.56	13.03	16.84	14.96	13.90	13.47	13.23
	75% Quantile	15.78	16.65	17.27	18.15	20.45	19.27	18.45	18.18	18.21
pH, s.u.	**N**	720	720	719	717	718	718	699	699	679
	Mean	7.73	7.81	7.98	7.69	7.71	7.47	7.59	7.66	7.76
	Median	7.70	7.74	7.94	7.69	7.73	7.48	7.58	7.67	7.74
	SD	0.11	0.21	0.18	0.09	0.14	0.14	0.06	0.13	0.09
	25% Quantile	7.64	7.64	7.82	7.63	7.60	7.45	7.55	7.57	7.70
	75% Quantile	7.81	7.93	8.10	7.76	7.81	7.52	7.62	7.76	7.81
Cl, mg/L	**N**	60	60	60	172	180	180	169	178	180
	Mean	80	871	1460	1689	1102	1389	1361	1452	1445
	Median	78	1012	1703	1795	1155	1465	1432	1526	1511
	SD	38	311	603	367	209	288	250	270	258
	25% Quantile	55	522	669	1308	964	1212	1148	1260	1224
	75% Quantile	100	1088	1948	1946	1260	1581	1545	1643	1624
Total Ammonia	**N**	58	59	59	172	180	180	170	179	180
mg N/L	**Mean**	0.10	0.13	0.39	5.11	9.98	7.65	6.04	5.65	4.74
	Median	0.10	0.13	0.38	5.02	10.00	7.77	6.04	5.49	4.76
	SD	0.06	0.05	0.24	1.78	1.47	1.60	1.44	1.52	1.58
	25% Quantile	0.08	0.10	0.16	3.83	8.89	6.49	4.95	4.59	3.60
	75% Quantile	0.10	0.17	0.58	6.10	11.08	8.73	7.01	6.70	5.84
Unionized Ammonia,	**N**	58	59	59	171	180	180	166	176	172
mg N/L	**Mean**	0.00	0.00	0.02	0.07	0.19	0.07	0.07	0.08	0.08
	Median	0.00	0.00	0.01	0.07	0.19	0.07	0.07	0.08	0.07
	SD	0.00	0.00	0.02	0.03	0.06	0.02	0.02	0.03	0.04
	25% Quantile	0.00	0.00	0.01	0.05	0.13	0.06	0.05	0.05	0.06
	75% Quantile	0.00	0.01	0.02	0.09	0.24	0.09	0.09	0.10	0.09

Appendix 2-4: Mean 15-Day Water Chemistry

Error bars represent the 95% confidence interval.



Appendix 2-5: Mean 30-Day Water Chemistry

Error bars represent the 95% confidence interval.